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# JOURNAL OF GENETICS

EDITED BY

W. BATESON, M.A., F.R.S.

DIRECTOR OF THE JOHN INNES HORTICULTURAL INSTITUTION

AND

R. C. PUNNETT, M.A., F.R.S.

ARTHUR BALFOUR PROFESSOR OF GENETICS IN THE UNIVERSITY OF CAMBRIDGE

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# CONTENTS

## No. 1 (April, 1924)

PAGE

S. A. ARENDSSEN HEIN. Studies on Variation in the Mealworm, <i>Tenebrio molitor</i> . II. Variations in Tarsi and Antennae. (With Nineteen Text-figures) . . . . .	1
A. S. PARKES. Studies on the Sex-ratio and related phenomena. II. The influence of the age of the mother on the Sex-ratio in Man	39
✓ F. L. ENGLEADOW. Inheritance in Barley. III. The Awn and Lateral Floret ( <i>continued</i> ): Fluctuation: a Linkage: Multiple Allelomorphs. (With Eight Text-figures) . . . . .	49
H. MUNRO FOX. Note on Kammerer's experiments with <i>Ciona</i> concerning the inheritance of an acquired character. (With Plate I)	89
Dr A. M. FREDERIKSE. Rudimentary Parthenogenesis in <i>Tenebrio molitor</i> L. (With Plate II and Six Text-figures) . . . . .	93
EDITH R. SAUNDERS. Further studies on Inheritance in <i>Matthiola incana</i> . I. Sap colour and surface character . . . . .	101
W. B. CROW. Variation and hybridization in <i>Isokontae</i> and <i>Akontae</i> in relation to classification . . . . .	115

## No. 2 (August, 1924)

A. E. WATKINS. Genetic and Cytological Studies in Wheat. I. (With Seventy-seven Text-figures) . . . . .	129
J. BRONTË GATENBY and F. W. ROGERS BRAMBELL. Notes on the Genitalia of a Crowing Hen. (With Four Text-figures) . . . . .	173
IRMA ANDERSSON. Structural Mosaics and Inheritance of Variegation in <i>Barbarea vulgaris</i> . (With Plates III—VIII) . . . . .	185
JAMES P. KELLY. Seed Progeny of a Potato with faintly coloured Tubers . . . . .	197
E. J. COLLINS. Inheritance of the Colour Pattern of King Edward Potato . . . . .	201
F. W. DRY. The Genetics of the Wensleydale Breed of Sheep. I. The occurrence of Black Lambs—an examination of Flock Records . . . . .	203
F. W. DRY. Sex ratio for two Chalcid Egg Parasites of the Coffee Bug ( <i>Antestia lineaticollis</i> ) . . . . .	219

	PAGE
W. E. CASTLE. Genetics of the Japanese Rabbit . . . . .	225
R. C. PUNNETT. On the "Japanese" Rabbit . . . . .	231
STEFAN KOPEĆ. Studies on the Inheritance of the Weight of New-born Rabbits. (With Two Text-figures) . . . . .	241
W. E. AGAR. Experiments with certain Plumage Colour and Pattern Factors in Poultry . . . . .	265

## No. 3 (December, 1924)

V. ISSAYEV. Researches on Animal Chimaeras. (With Plates IX—XVII and Six Text-figures) . . . . .	273
K. TJEBBES. Crosses with Siamese Cats. (With Plate XVIII) . . . . .	355
J. A. FRASER ROBERTS. Colour Inheritance in Sheep. I. Black Colour and Badger-face Pattern in Welsh Mountain Sheep. (With Plates XIX—XX) . . . . .	367
G. D. KARPECHENKO. Hybrids of ♀ <i>Raphanus sativus</i> L. × ♂ <i>Brassica oleracea</i> L. (With Plates XXI—XXII and Three Text-figures) . . . . .	375
W. B. CROW. Variation and Species in <i>Cyanophyceae</i> . (With Eight Text-figures) . . . . .	397

# CORRIGENDA.

JOURNAL OF GENETICS xiii, 1923, No. 2, p. 236.

By error in copying, certain numbers were misplaced in the 4 left-hand columns of the upper Table, and in the 3 left-hand columns of the lower Table. Below are given the corrected figures with the class-Totals correspondingly corrected. The families affected were not specially referred to in the text, and the corrections create no disturbance in the family-Totals or in the general argument.

54	55	58	59	Totals
5	18	17	2	192
10	13	11	12	200
4	10	17	3	171
9	17	11	11	161
13	22	20	10	199
14	16	18	9	174
10	11	12	6	149
7	12	16	6	181

60	63	64	Totals
11	11	10	74
10	8	6	63
16	4	5	61
9	7	11	65
18	13	15	85
10	12	12	85
8	12	11	72
13	14	14	79

*Ibid.* p. 252, in description of Fig. 6, for *bGL*, read *bgL*.





# STUDIES ON VARIATION IN THE MEALWORM, *TENEBRIO MOLITOR.*

## II. VARIATIONS IN TARSI AND ANTENNAE.

By S. A. ARENDSSEN HEIN.

(With Nineteen Text-figures.)

### CONTENTS.

	PAGE
A. Introduction . . . . .	1
B. Non-hereditary anomalies . . . . .	3
<i>a.</i> In the antennae . . . . .	3
1. Reduced number of joints . . . . .	3
2. Increased number of joints . . . . .	5
3. Other variations . . . . .	6
4. The variation At Px . . . . .	6
<i>b.</i> In the tarsi . . . . .	7
1. Reduced number of joints . . . . .	7
2. Increased number of joints . . . . .	8
C. Geno-variations . . . . .	10
<i>a.</i> The strain At c . . . . .	10
<i>b.</i> The strain At 10/10 . . . . .	19
<i>c.</i> The strain T a . . . . .	27
<i>d.</i> Crosses of T a $\times$ At 10/10 . . . . .	29
<i>e.</i> Crosses of T a $\times$ At c . . . . .	30
D. Discussion . . . . .	30
E. Summary . . . . .	35
F. Literature . . . . .	38

### A. INTRODUCTION.

TAKING the word in a general sense, the variations to be treated in this paper are, with a few exceptions, meristic in their nature, because they deal with a decrease or increase in the normal *number* of partitions of segmented organs, viz. the tarsi and antennae. Whether they are meristic in the narrower sense which Bateson, who introduced this term in science, ascribed to it, we will discuss when the facts have been given.

*Increase* in number of joints is in the tarsi a relatively rare occurrence; still these deviations will always be found when a few thousands of individuals are examined.

In the antennae it was observed only four times amongst the great number of individuals investigated.

## 2      *Variation in the Mealworm, Tenebrio molitor*

*Decrease* in number in the tarsi as well as in the antennae is much less rare and in most cases is found on one side only, in a way suggestive of developmental modification unlikely to produce genetic consequences. In other cases, however, when such deviations similarly occurred on both sides, it was possible, by a rigid selection and inbreeding, to obtain strains in which the anomaly was maintained, segregating in  $F_2$  when crossed with normal individuals. But the symmetry or asymmetry of distribution is no certain indication whether the abnormality is a hereditary deviation or not.

An actual breeding test may give results altogether contrary to expectation. We shall see that in strains showing a hereditary reduction in their antennal or tarsal joints, these reductions may not seldom concern only one organ, either *one* antenna (the other being normal), or *one* tarsus (the other five legs remaining unaffected).

With this experience in mind, I learned to be careful and not to pre-judge a given case by classing it beforehand as a fortuitous modification. Hence the classification of the observed variations as non-transmissible anomalies, and genetic variations, must, as regards the former, be regarded as provisional. Only a *few* of them have been studied in their genetic behaviour, and on account of the negative results obtained, analogous variations have been classed with them.

It is worth mentioning that, though the number of beetles investigated on deviations has been great, we have never met with cases of *Homoeosis*, a phenomenon which Bateson adequately defines as a variation by which "something has been changed into the 'likeness of something else'" [(2), p. 85], for instance, an antenna into a foot; a leg into a wing, etc. [(2), pp. 147-155].

The three genotypes treated in this paper seem to be very rare, as, in the far more than 100,000 beetles already examined, they appeared as a few individuals only once in *one* culture, and at the same period. Their sudden appearance, their hereditary nature, the rareness of their occurrence, the loss of a character which their parents possessed, all this suggests that they might be called mutations in the sense of de Vries. Morgan and his school have done this for the new characters appearing in their *Drosophila* experiments. However, I think that this term should not be applied to every novelty which turns up in our cultures, unless other causes of their appearance are excluded.

Until this requirement is fulfilled, I prefer to use the more neutral expression *genovariation*, which makes no assumption as to the modus of their creation.

What has been communicated here about the above-mentioned genovariations marked by the initials At c, At 10/10, and T a, ought to be considered as preliminary.

A few crosses between the strains At c  $\times$  T a have produced results which require to be controlled on a larger scale before definite conclusions can be drawn.

Also, on account of the more or less similar deviations in the phenotypes At c and At 10/10, we met with great difficulties in recognising their combinations in the  $F_2$  of a cross of them.

On another occasion we have to return to this and to some other points about which we are in doubt, when the experiments in progress have given the answer to the questions we have put to them.

## B. NON-HEREDITABLE ANOMALIES.

### *a. In the antennae.*

#### 1. *Reduced number of joints (antennae).*

It should be remembered that the normal number of joints is eleven (Fig. 2). Reductions in the antennae are not so very rare. They appear in all sorts of conditions.

That a reduction is often caused by a fusion of the joints is indicated by the abnormal length of the joint, or more clearly by small contractions which correspond with the number of joints fused, or still more conspicuously by a trace of a suture. In Figs. 1 to 11 a selection out of many more different conditions which came under observation is shown.

Fig. 1a, 1b, 1c. Different cases of 12 jointed antennae.

„ 2 = the normal condition (11 joints).

„ 3 = 10 joints (8th and 9th fused).

„ 4 = 9 „ (8th and 9th, and 10th and 11th fused).

„ 5 = 9 „ (4th and 5th, and 6th and 7th fused).

„ 6 = 8 „ (8th to 11th fused).

„ 7 = 7 „ (4th and 5th, and 8th to 11th fused).

„ 8 = 6 „ (6th to 11th fused).

„ 9 = 5 „ (4th to 10th fused).

„ 10 = 4 „ (4th to 11th fused).

„ 11 = 3 „ (3rd to 11th fused).

We notice that with exception of the scapus and pedicellus, in which no fusion has ever been observed, all others may lose their independence and be united partially or wholly with one or more of the next following.

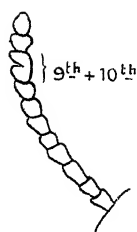


Fig. 1a

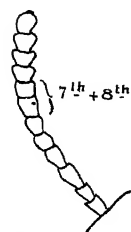


Fig. 1b

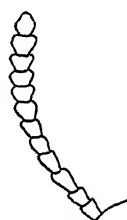


Fig. 1c

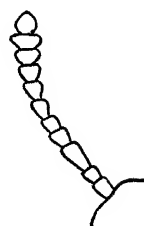


Fig. 2

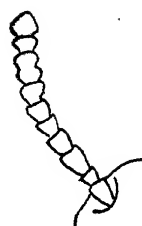


Fig. 3

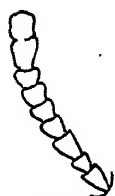


Fig. 4

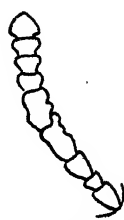


Fig. 5

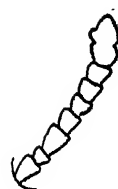


Fig. 6

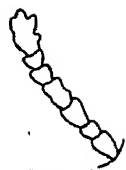


Fig. 7

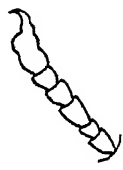


Fig. 8



Fig. 9



Fig. 10



Fig. 11

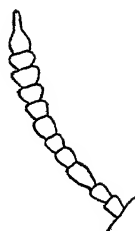


Fig. 12



Fig. 13

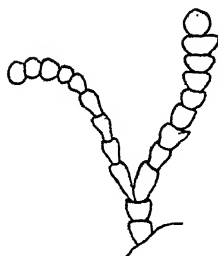


Fig. 14

That such reductions as are shown in Fig. 5 (9 joints), Fig. 6 (8 joints), Fig. 7 (7 joints), Fig. 9 (5 joints), Fig. 11 (3 joints) are not caused by loss of the peripheral joints is indicated by the form of the terminal joint, which, in its apical part, retains the conical shape, which form is specific for *T. molitor*.

As the results studied in the few crosses made with these reductions (mostly occurring only in *one* antenna) were negative, they are evidently to be regarded as disturbances in development which may nevertheless not rarely affect both antennae similarly.

Fusion by which the fused joints form a spiral, as we have repeatedly noticed in the body of the larvae [(1a), p. 230, Figs. 2, 6, 12 a.o.] and as Bateson in his *Materials* mentions for *Lumbricus* [(2), pp. 157-158], we never met in the fused joints of the antennae.

## 2. Increased number of joints (antennae).

In rare cases the number of joints is increased by one. Since my former paper I have noticed two fresh examples of this, and in each *both* antennae exhibited 12 joints (Fig. 1b).

Of these four cases three were females and one male.

We made three crosses with the following results:

Reference	No. of cross	Parents		$F_1$ normal	$F_2$		$F_3$ normal	Total	
		♂	♀		normal	At 12/12		normal	At 12/12
BL. 37	1	At 12/12	At normal	49	50	—	—	99	—
„ 106	2	At normal	At 12/12	16	63	2 <sup>1</sup>	117	196	2
„ 252	3	At normal	2 ♀♀ At 12/12	32	164	—	—	—	—
Totals				97	277	—	—	—	—

<sup>1</sup> 1 ♀ = At 11/12; 1 ♀ = At 12/12.

In both antennae of No. 1, No. 2 and the right antenna of one of the females of No. 3, the new joint arose by a division of the 7th joint. This could be ascertained first by the constriction between the 7th and 8th joints being only indicated, though the suture between them was clear enough, and secondly by the large size of the 7th and 8th which together was longer than the previous one (6th) or the following (9th) (Fig. 1b).

In the left antenna of No. 3 it was not clear where the extra joint was formed as all the 12 joints looked quite normal (Fig. 1c). In the second female of No. 3 the extra joint could be seen in *status nascendi*. The 9th joint was double the size of the 8th or 10th and showed at one side a deep constriction or partial division (see Fig. 1a).

The tarsi of this beetle had a supernumerary joint in the 1st and 3rd legs, having also the composition T 6/6, 5/5, 5/5.

## 6 *Variation in the Mealworm, Tenebrio molitor*

In all three crosses  $F_1$  (97 beetles) was normal, as also were the  $F_2$  beetles of cross No. 1 and of cross No. 3. Among 65  $F_2$  beetles of cross No. 2 in two ♀ individuals the anomaly reappeared: in one of them the left antenna was normal, the right one had 12 joints; in the other ♀ both antennae were 12-jointed. Can it be a mere chance accident that two beetles of the 65 show this anomaly again, which is so very rare when looked for in a population at random?

### 3. *Other variations (antennae).*

Besides the variations in the number of the antennal joints, others are observed in which, the number being normal, one or more joints are abnormally enlarged or diminished.

A bifurcation of one of the antennae was observed twice, the accessory antenna took its origin from the apex of the second joint (Fig. 13).

These variations, some of which (when the anomaly appeared in both antennae) have been tested as to their heredity, but with negative results, are for the present of no special interest.

### 4. *The variation At Px (antennae).*

A peculiar antennal deviation remains to be mentioned upon which we must enter into some detail. The terminal (11th joint), whose shape and relative dimensions is of taxonomic value in the different species of *Tenebrio*, was drawn out in a long point assuming the form of a Pixavon bottle<sup>1</sup> to be seen in every hair-dresser's shop. We will henceforth call this anomaly Px. This deviation was repeatedly observed, but appeared only in *one* culture of our measurements series. That culture contained beetles whose ancestors in ten generations were selected for a length of the pupae between 17 and 18 mm.; it was the 17/18 group of the above-mentioned series. That the anomaly was never noticed in our other *molitor* cultures, but again and again only in the 17/18 group I consider as merely accidental, because the same deviation was lately, though only once, observed also in a population of *T. obscurus*.

When the first beetle appeared we crossed it with a normal individual. Among the 66  $F_2$  beetles two ♂♂ showed the anomaly again; in one the joint was only pointed, the other had the pure Px shape. The  $F_1$  gave 20 beetles of which one ♂ had pointed antennae. The 63  $F_3$  beetles were all normal.

Meanwhile, as more beetles showing the same deviation presented themselves, we obtained an  $F_1$  generation derived from parents in which the anomaly was bilateral and well-developed (Fig. 12).

<sup>1</sup> English readers, by reference to Fig. 12, may see the shape thus named. Ed.

Of the 50  $F_1$  beetles

29 individuals or 58 %	had both antennae Px
10 "	20 % had one antenna Px, the other sharply pointed
1 "	2 % " " " normal
4 "	8 % had both antennae pointed

---

So 44 " 88 % were abnormal. The remaining 13 % perfectly normal

From the  $F_1$  15 beetles, Px in both antennae, and 16 individuals, Px in one antenna and sharply pointed in the other, were kept and mated.

Of 33 beetles in  $F_2$

11 or 33 %	had both antennae Px
1 " 3 %	had one antenna Px, the other pointed
1 " 3 %	" " " normal
6 " 18 %	had both antennae pointed
3 " 9 %	had one antenna pointed, the other normal

---

So 22 " 66 % were abnormal

The remaining 11 beetles, or 33.3 per cent., were perfectly normal.

My further experiments have not proceeded far enough for me to say more of this anomaly.

A series of crosses with normal beetles has been started; likewise the 11 normals of the above-mentioned  $F_2$  have been kept separate for an  $F_3$ . The fact that in the only cross, mentioned above and studied till the  $F_3$ , no segregation in a definite ratio was observed, gives an indication that the Px antenna is only a simple non-hereditary modification. The high percentage of 88 per cent. in the  $F_1$ , however, descending from a cross in which all  $P$  parents were Px, leaves the possibility open that by a continued sharp selection and in-breeding a strain may be obtained pure for the presence or absence of a factor underlying this anomaly, if such a factor exists.

#### *b. In the tarsi.*

##### *1. Reduced number of joints (tarsus).*

In my former paper [(1a), pp. 258-261], I already mentioned the reductions observed in the tarsal joints and gave figures of different cases in which the tarsus consisted of four, three, two or only one joint.

To what I have said about these reductions the following may be added. At first, when I possessed only the above-named one-sided anomalies, I studied some of them in their hereditary behaviour but without any result. I did not pay more attention to them, except in those cases in which the anomaly appeared in both legs of the same pair. And these conditions, too, were not studied any more since we got the two



hereditary strains to be treated in the next chapters. For the moment nothing can be said as to the cause of these, mostly uni-, sometimes bilateral, meristic reductions.

For reasons which will be given subsequently it is, however, certain that even a *partial* reduction in *one* of the six tarsi may be called forth by the presence (or absence) of a genetical factor. This factor may influence the normal course of ontogenetic division in the tarsi of *all six* legs when, by a rigid selection and inbreeding, purity in respect of such a factor is obtained, giving it a chance to manifest itself in a changed phaenotypical appearance of the individuals pure for it.

## 2. *Increased number of joints (tarsus).*

On p. 260 of my former paper I mentioned the four cases I found showing an increased number of the tarsal joints. Since then, we went on searching for such anomalies. We examined in total 42,487 beetles; 69 of them showed an increased number in one, in two, or in all the three pairs of the legs. So on an average we found one case for 615 beetles examined. Of course such a proportion has little significance beyond giving an idea of the frequency of occurrence.

We effected 14 crosses; the conditions found in the parents, and the results in the subsequent generations, as far as they are kept, are given in Table I.

In crosses Nos. 2, 4, 5, 7, 8, 9 and 10 one parent was abnormal, the other normal.

In cross No. 1 both parents abnormal.

In cross No. 3 of the four parents three were abnormal.

In cross No. 6 a mating of a mixed collection of abnormals and normals, the former found in different populations in which the abnormal ♀♀ had already copulated with males of unknown descent.

In crosses Nos. 11, 12 and 13 one parent abnormal, the other an individual of the strain T a (see p. 27).

In cross No. 14 were brought together *normal* beetles obtained from the  $F_1$  of No. 6; the  $F_2$  of No. 3; and the  $F_3$  of No. 2.

Regarding the abnormals in the  $F_1$  and  $F_2$  the following must still be mentioned.

In the crosses No. 2, No. 4, No. 7 and No. 8 the abnormals found in the  $F_1$  and  $F_2$  have been kept together with the normals of that generation to produce eggs ( $F_2$  or  $F_3$ ). In cross No. 9 the two abnormals obtained in the  $F_2$  were of different sex and appeared at the same time. They were kept separate from the normals which were discarded; the 38  $F_3$  beetles of this cross originate also from these two abnormals.

In cross No. 5 the  $P$  parents originate from the  $F_2$  of cross No. 2; so the  $F_1$  of cross No. 5 is an  $F_3$  of cross No. 2. For this reason we kept no  $F_2$  of cross No. 5.

TABLE I.

Reference	No. of cross	Parents		$F_1$		$F_2$		$F_3$		Total $F_1-F_3$	
		♂	♀	Normal	Abn.	Normal	Abn.	Normal	Abn.	Normal	Abn.
BL. 32	1	5/5, 6/6, 5/5	5/5, 5/5, 5/5	19	1 <sup>1</sup>	—	—	—	—	19	1
„ 28	2	Normal	6/6, 6/6, 5/5	23	1 <sup>2</sup>	63	3 <sup>3</sup>	102	1 <sup>4</sup>	188	5
Pt iii. 5	3	1. Normal 1. 5/5, 5/5, 5/5	2. 5/5, 5/5, 5/5	33	—	99	1 <sup>5</sup>	—	—	132	1
„ 58	4	Normal	6/6, 6/6, 5/5	55	1 <sup>6</sup>	49	1 <sup>6</sup>	50	—	154	2
„ 71	5	„	6/6, 6/6, 5/5	50	—	—	—	—	—	50	—
Pt iii. 5(2)	6	Diff. abnormal	Diff. abnormal	239	3 <sup>7</sup>	—	—	—	—	239	3
„ 107	7	Normal	6/6, 6/6, 5/5	19	1 <sup>8</sup>	28	1 <sup>9</sup>	2	—	49	2
„ 109	8	„	6/6, 6/6, 5/5	13	1 <sup>10</sup>	63	—	43	—	119	1
„ 110	9	„	6/6, 6/6, 5/5	25	—	50	2 <sup>11</sup>	38	—	113	2
„ 180	10	„	6/6, 6/6, 5/5	8	—	98	1 <sup>12</sup>	—	—	106	1
„ 105	11	T a	6/6, 6/6, 5/5	22	—	59	1 <sup>13</sup>	—	—	81	1
„ 108	12	5/5, 5/5, 5/5	T a	12	—	67	—	—	—	79	—
„ 157	13	T a	5/5, 5/5, 5/5	43	—	84	—	—	—	127	—
Total	—	—	—	561	8	660	10	235	1	1456	19
Pt iii. 5(3) bis	14	Normal	Normal	207	—	—	—	—	—	207	—

<sup>1</sup>=5/5, 5/5, 5/5;    <sup>2</sup>=5/5, 5/5, 5/5;    <sup>3</sup>=6/6, 5/5, 5/5;    <sup>4</sup>=5/5, 6/6, 5/5;    <sup>5</sup>=6/6, 6/6, 5/5;  
<sup>6</sup>=6/6, 6/6, 5/5;    <sup>7</sup>=1 bt=6/6, 6/6, 5/5; 2 bt=5/5, 6/6, 5/5;    <sup>8</sup>=6/6, 6/5, 5/5;    <sup>9</sup>=6/6, 6/6, 5/5;  
<sup>10</sup>=5/5, 5/5, 4/5;    <sup>11</sup>=1 bt=5/5, 5/4, 5/5; 1 bt=6/6, 6/6, 4/4;    <sup>12</sup>=5/5, 6/5, 5/5;    <sup>13</sup>=5/5, 6/6, 4/4.

In my former paper [1*a*], p. 261] I mentioned that the frequency of the reappearance of the anomaly in such crosses, as are given in Table I, was not greater than in any population taken at random. This statement is incorrect; it was based on a general impression of the experiments still in progress.

If we determine the average ratio between the normals and abnormalities in the  $F_2$  from those crosses (Nos. 2, 4, 7, 8, 9, 10, 11, 12 and 13) in which one of the  $P$  parents was abnormal we find that amongst the 570 beetles nine in all were abnormal, that is, one in 64. One might feel inclined to see in this a trihybrid cross, the result of the co-operation of three factors which, when simultaneously present, affect the tarsi in such a way that one pair (crosses No. 3, No. 12 and No. 13) or two pairs (♂ of cross No. 1) or three pairs (crosses No. 2, No. 4 and No. 7) of them, are increased by one joint.

If, however, we take no notice of the general average (which might be quite misleading) but consider the cases separately and compare them mutually, there is very little suggestion, if any, that genetical factors have anything to do with this anomaly.

## C. GENOVARIATIONS.

a. *The strain At c.*

In a mixed culture started as an experiment on the colour of the beetles, there appeared within a few days two beetles of opposite sexes which showed a reduction, a shortening and a broadening of the antennal joints.

These two beetles, which were the only ancestors of our strain, exhibited the following peculiarities.

*The male.* Both antennae consisted of ten joints, the 4th and 5th were fused without any trace of suture between them, only the abnormal length of the 4th joint gave indication that the next segment was fused with it. This 4th joint was as long as the 3rd, which in all *Tenebrio* species is of taxonomic value and is always the longest of all. The other joints (6th to 10th) were in both antennae all clearly distinct but showed the peculiarity that they were compressed in the axial line, so that the antenna as a whole gave the impression of being shortened and the joints broadened.

The number of the tarsal joints was normal in this parent ( $\sigma$ ).

*The female.* In the  $\varphi$  three or four of the terminal joints of both antennae were wholly or partially fused; only nine joints could with certainty be distinguished; the compressed condition of the antennal joints was in the  $\varphi$  more conspicuous. The first and second pairs of legs were normal. In the third pair the tarsus of the left leg counted three joints instead of four, an indication of a fusion of the 3rd and 4th joints was still to be seen.

In  $\sigma$  and  $\varphi$  *all* the legs were shorter than in a normal beetle, they lacked that slenderness which a well-developed limb exhibits.

Not the *number* of the antennal joints but the *shortened* and *broadened* condition of the antennae as a *whole*, whether or not accompanied by a partial or complete fusion of two or more joints, is the principal point by which the obtained strain is characterised.

We will henceforth call this race At c (Antennae compressed).

Besides the anomalies in antennae ( $\sigma$  and  $\varphi$ ) and tarsi ( $\varphi$ ) there are two other deviations to be mentioned in this original pair which, to the uninitiated, are less conspicuous; they can only be truly appreciated by one who is thoroughly acquainted with the normal type. The deviations consist first in a rounding of the sharp angles of the scutellum and the front-horns of the prothorax, secondly in a relatively broader prothorax, in proportion to its length.

For a right understanding a more precise explanation is wanted:

*Scutellum*. This organ is in *T. molitor* as a rule pentagonal (Fig. 15). In both parents the points *a*, *b*, *c* were rounded off (Fig. 16) and came very near to the condition found in *T. picipes*, which differs from all other species by its semicircular scutellum.

*Front-horns*. The proximal edge of the prothorax shows in the normal beetle at *d* and *e* (Fig. 15) (where it meets the side-edges) two more or less sharp points which are directed to the front.

These points I have called the front-horns of the prothorax. In both parents these front-horns were also rounded off (Fig. 16) and in the ♂ more than in the ♀.

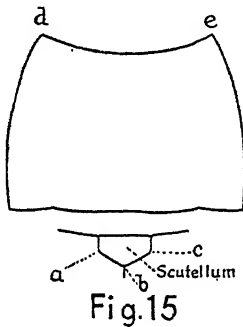


Fig.15

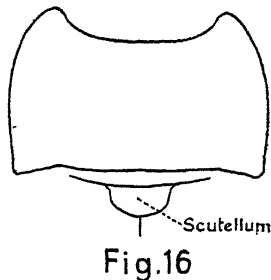


Fig.16

*Breadth of the prothorax*. The length and breadth of the prothorax play an important part in the descriptions and diagnoses of the *Tenebrio* species. In these systematic descriptions no mention is ever made of actual dimensions. Only the relation, the index between length and breadth as judged by the eye, is referred to.

Besides the most common type of this relation, there are others in *T. molitor* in which the prothorax is relatively much longer (approaching the condition found in *T. obscurus*), and these variations occur very often in contrast to the broader prothorax which is rare, though common in *T. syriacus*.

In our *P* beetles the ♀ showed a prothorax of a more than usual breadth; in the ♂ the breadth was a trifle more than the common type.

The facts that both beetles originated from the same culture and were obtained at nearly the same time, further that they both exhibited similar variations of scutellum, front-horns and antennae, make it probable that their origin must be sought in the same sort of geno-variation.

From the cross of these two beetles, four generations were kept. For the propagation of  $F_2$  I selected only those  $F_1$  beetles of which the antennae

## 12 *Variation in the Mealworm, Tenebrio molitor*

were reduced or shortened and which at the same time possessed a prothorax broader than usual. In  $F_2$  the selection was carried on in two directions, viz.

*Group A* with a broad prothorax.

*Group B* with abnormal antennae.

The  $F_3 A$  beetles descend from 14  $F_2$  parents, all with a conspicuous broad prothorax, but of which four individuals had abnormal antennae. The  $F_3 B$  descends from six  $F_2$  parents, all with abnormal antennae, but of which four beetles had a normal prothorax.

As nearly all the  $F_3 A$  and  $F_3 B$  beetles had the antennae reduced and the prothorax broad, we did not continue this selection. Moreover, it had not been carried out in a strictly rigorous manner because I feared to obtain an insufficient progeny. The eggs obtained from these two  $F_3$  groups were brought together in one jar representing the  $F_4$  of this line.

The results of these four generations are tabulated as follows:

TABLE II.

Generation	Beetles obtained in total	Abnormal antennae and prothorax		Normal antennae and prothorax		Abnormal in total		Abnormal in %			No. of beetles of which 2nd leg had 4 joints
		Nor.	Abnor.	Nor.	Abnor.	In anten.	In prothor.	In anten.	In prothor.	In antennae and prothor.	
	1	2	3	4	5	2 and 3	3 and 5	6	7	8	9
$F_1$	29	9	14	2	4	23	18	79	62	48	1
$F_2$	50	3	10	20	17	13	27	26	54	20	3
$F_3^A$	15	—	13	—	2	13	15	86	100	86	—
$F_3^B$	35	6	28	—	1	34	29	97	82	80	5
$F_4$	54	16	32	5	1	48	33	88	61	59	4
Totals	183	34	97	27	25	131	122	71	66	53	13

If we leave the form of the prothorax out of consideration, and restrict our attention to the reduced and shortened antennae, we see that 79 per cent. of the  $F_1$  beetles exhibited the anomaly in that organ.

As we said, out of the  $F_1$  we selected to breed  $F_2$  only those beetles in which the antennae were abnormal; the result was that of the 50  $F_2$  beetles only 13, or 26 per cent., had abnormal antennae. Notwithstanding that the selection amongst the  $F_2$  beetles kept to breed  $F_3 A$  was *not* carried out with respect to abnormal antennae (ten of the 14  $F_2$  parents were normal) but with respect to a broad prothorax, still 13 of the 15  $F_3 A$  beetles, i.e. 86 per cent. had abnormal antennae.

In  $F_3 B$ , which descended from  $F_2$  beetles all with abnormal antennae, the percentage amounts to 97 per cent. abnormal.

In  $F_4$ , descending from the 15  $F_3 A$  and 35  $F_3 B$  beetles of which only

three were normal, the percentage of abnormal antennae comes to 88 per cent.

The cause of this irregularity in the number of beetles showing the anomaly in the different generations is not quite clear; in particular the low rate in  $F_2$  is strange when we know that their 14 parents all had abnormal antennae. The same can be said of the reverse case, the  $F_3 A$ , with its high percentage of 86 per cent. abnormal, whereas ten of the 14  $F_2$  parents possessed normal antennae.

Still there is something to be noticed which may furnish an explanation.

If we study the anomaly carefully, the first thing we notice is that the condition varies in different individuals. In the one extreme, all the joints keep their independence, the normal number 11 being kept, though anybody familiar with this material will not hesitate in declaring the antennae abnormal through the shortening and broadening of their joints, which produces the impression that the whole antenna has been compressed in the axial line ( $a$ , Fig. 17). In the other extreme only seven or eight joints can be counted, caused by the total fusion of members which are separated by unfused joints ( $o$ , Fig. 17).

Between these two extremes different conditions are noticed in which one, or two, or three joints are either wholly or partially fused with their neighbour ( $d-n$ , Fig. 17).

In short, the abnormality is subjected to a great variability. If one factor causes these abnormal appearances, this factor must affect the phenotype in different degrees. Its influence may be so slight that the antenna scarcely differs from the normal. On account of the 86 per cent. abnormal antennae in the  $F_3 A$  beetles, descending from 14 parents of which ten were normal, I presume that the said factor may still be present without affecting the antennae at all. In the two other strains treated in the next chapters we shall meet with similar occurrences.

The different characters which this strain exhibits, viz.: the shortened or fused antennal joints, the pseudo-circular scutellum, the rounding of the front-horns, the broad prothorax, the shortening, sometimes reduction, of the tarsal joints, all work in the same direction, namely a compression in the axial lines, giving the beetle the thick-set appearance characteristic of this strain. These characters are not always simultaneously present, but it is by no means so very rare that all five peculiarities can be noticed in one individual. Three of them (reduced antennae, rounded front-horns, broad prothorax) commonly appear together; besides, abnormal antennae are, as a rule, accompanied by

## 14 *Variation in the Mealworm, Tenebrio molitor*

a compact condition of the tarsal joints, and exceptionally by a reduction in their number.

In the above-mentioned four generations, in which only the tarsus of the second and third leg was observed, we noticed this reduction 13 times, all in the second leg, of the 183 beetles investigated in all (see Table II, column 9).

I am inclined to believe that only one genetical factor is responsible for the above-named characters, because, though it influences different organs, it affects these organs in a similar way, viz. they lose their slender appearance. But of course it is also possible that several segregable factors cause these different deviations, and that their frequent co-existence is brought about by a linkage which is not easy to break.

Besides crossing with the normal type, the next work to be done was to see what could be obtained by more rigorous selection and in-breeding.

Unfortunately the  $F_5$  yielded only a few beetles, all of the same sex, so that the original strain died out. At that moment I knew yet nothing about the nature of these anomalies, in fact I had very little confidence that they represented a genovariation. On account of the strange results in  $F_2$ ,  $F_3 A$  and  $F_3 B$  (Table II), I felt more inclined to see in the behaviour of this anomaly of the antennae something analogous to the case of the torsion of the stems in *Dipsacus* mentioned by de Vries.

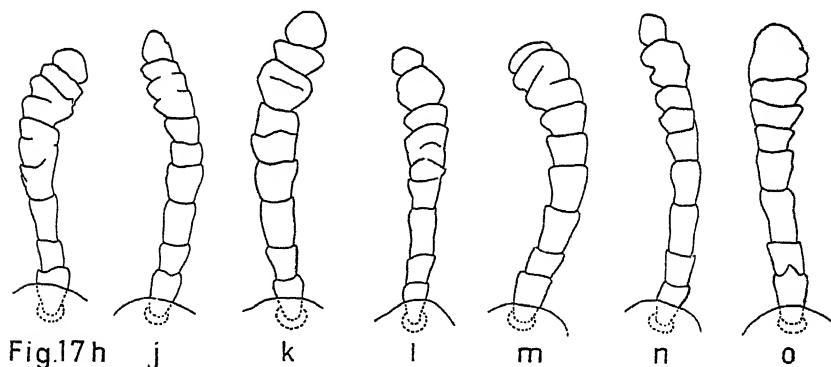
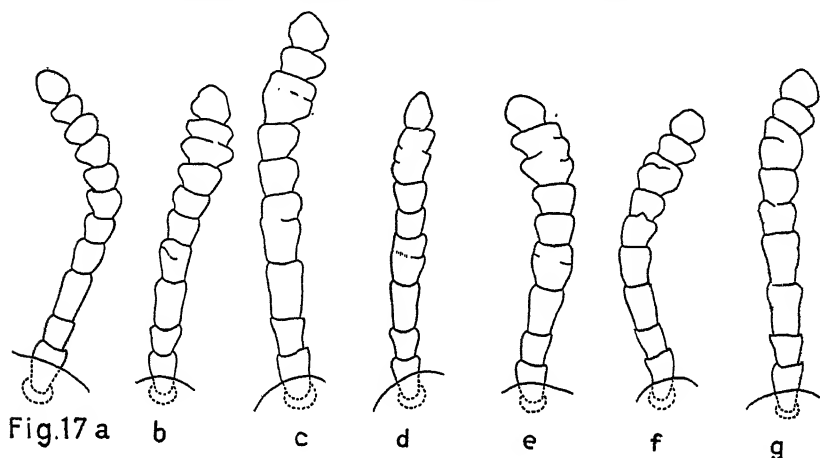
Still I had previously already started ten crosses, and to my great satisfaction I saw the lost anomaly reappear in  $F_2$  in such a ratio to the normal ones, that any doubt regarding a monohybrid segregation, could not be maintained.

We mated the abnormal  $F_2$  individuals of these ten crosses together in order to reconstruct first of all the pure strain which, as was stated, had died out in  $F_5$ . They produced 78 individuals, all, without exception, having abnormal antennae; 38 showed a broad prothorax; in 13 beetles a reduction of the tarsus by one joint was observed as follows:

Number of beetles	1st leg	2nd leg
9	—	<i>R</i> and <i>L</i>
1	—	<i>L</i>
1	<i>R</i> and <i>L</i>	—
1	<i>L</i>	—
1	<i>R</i> and <i>L</i>	<i>R</i> and <i>L</i>
<hr/> 13		

On p. 13 I suggested that the responsible factor may be present (or absent) without affecting the antennae at all. This was written down some time ago. Quite recently I observed a fact which actually offers

a proof of this supposition. After the culture had yielded the 78 beetles mentioned above, it was stopped and put outside the incubator on the table of the laboratory. This means that the temperature for the remaining larvae was lowered to about 17–20° C. By this, the pupation was abruptly stopped, and, so we took no further notice of it until after some time the pupae commenced to reappear in small numbers. Re-



membering that *all* the 78 beetles previously obtained were abnormal in their antennae, I noticed to my great astonishment that most of the beetles appearing now, had normal antennae.

They could, however, at once be recognised as belonging to this strain (At c) by the want of slenderness in their legs, especially the tarsus, or by the form of the prothorax, or by their rounded front-horns. After this had gone on for a while, we put the larval jar in the incubator again,



and whilst the beetles obtained from the next two or three collections still had mostly normal antennae, those obtained much later showed the anomaly in the old-fashioned way.

All this is not offered as an experiment on the influence of temperature, but as a chance observation needing further control. However, this fact supports my suggestion that the antennae are not always affected, but may be influenced by environmental conditions.

About the abnormal antennae we must enter into some detail. The compressed condition of the antennal joints often causes a partial or total fusion of two or more joints; this fusion may be observed in different members of the series, but as a rule the 1st, 2nd and 3rd remain intact. A fusion of the 3rd and 4th I never noticed, of the 4th and 5th often, but generally accompanied by a partial or complete fusion of the more peripheral members.

I specially emphasise this, as in the strain to be treated next, we shall meet with a case in which the fusion of the 4th and 5th joints is the rule but the other members are mostly not affected. In Fig. 17, *a-o*, various cases are shown, the outlines being made with a drawing prism.

In *a* we count 11 joints;

„ <i>b</i>	„	11	„	4th and 5th partially fused;
„ <i>c</i>	„	11	„	4th and 5th, and 8th and 9th partially fused;
„ <i>d &amp; e</i>	„	11	„	4th and 5th, and 8th to 10th partially fused;
„ <i>f &amp; g</i>	„	10	„	4th and 5th (?) wholly, 8th and 9th partially fused;
„ <i>h</i>	„	11	„	4th and 5th, 6th and 7th, and 8th and 9th partially fused;
„ <i>j</i>	„	10	„	7th to 9th partially, 10th and 11th wholly fused;
„ <i>k</i>	„	10	„	4th and 5th (?) wholly, 8th and 9th partially fused;
„ <i>l</i>	„	10	„	5th to 7th partially, 9th and 10th wholly fused;
„ <i>m</i>	„	10	„	4th and 5th (?) wholly, 8th to 10th partially fused;
„ <i>n</i>	„	9	„	4th and 5th (?) wholly, 9th and 10th wholly fused;
„ <i>o</i>	„	8	„	4th and 5th (?) wholly, 9th to 11th wholly fused.

If we disregard the slight constrictions and the other indications of the morphological nature of fused joints, and count as *one* joint every case in which no trace of a suture between the fused joints is to be seen, we may give some approximate idea how often different conditions have occurred in the 156 antennae of the above-mentioned 78 individuals. So taking only into account the *number* of joints and not the *way* in which this number came about, it may be stated that in most cases this reduction is the same in both antennae; but the number in one antenna not rarely differs from that in the other.

Even the antennae with the normal number of joints, 11, were not really normal, but showed the deformations characteristic of the strain, often accompanied by partial reduction. Though the other parts (pro-

thorax, etc.) are normal these specimens could be distinguished at a glance. We observed the following cases:

One antenna	8	the other	7 joints	No.
"	8	"	9 "	1
"	8	"	10 "	1
"	9	"	10 "	6
"	9	"	11 "	2
"	10	"	11 "	4
Both antennae	8 joints			1
"	9 "			16
"	10 "			34
"	11 "			12
				<hr/>
				78 beetles

This fact, in connection with the variations shown in Fig. 17, *a-o*, indicates that the actual *number* of independent joints in this strain (At c) is of secondary value.

Again, the compressed condition is the main point; the final result, a fusion of two or of three or of four joints, partial or even complete, is secondary, depending perhaps largely on the environment to which the pupae are exposed during development. In the beginning, when the anomaly was studied in the first two generations I had, of course, not yet reached this conclusion. At *that* time I named the At c strain At 10/10, on account of the frequent occurrence of the ten antennal joints, though that name properly belongs to the race to be treated next.

After becoming more thoroughly acquainted with the material, comparing it with the true At 10/10 strain, I learned their fundamental difference. It was a mere accident that both anomalies were found at nearly the same time though in different cultures (At 10/10 in June-August, 1918, and At c in September, 1918) and that both showed similar variations, viz. reductions in the antennal and tarsal joints.

In the first generations of the At c strain I regarded the presence of ten antennal joints as the principal character and looking back now, I see that what were given in Table II, columns 4 and 5 as "normal antennae" must be interpreted as abnormals in view of the compressed condition of the antennae, though 11 joints are present. It seems to me now very probable that the matter must be explained in this way, but I could not *then* mention it in my notes, and have therefore given the table in accordance with the facts and conceptions *then* recorded.

We will now mention the results of the crosses, tabulated in Table III.

In total 10 crosses were made, every cross consisted of one ♂ and one ♀.

TABLE III.

*Crosses of At c × normal.*

Reference	No. of cross	Beetles obtained in $F_1$				Beetles obtained in $F_2$				$F_2$ Ratio of normal: abnormal antennae	Prothorax		$F_2$ Ratio of normal: abnormal prothorax
		In total	At normal	At ab-normal	Pro-thorax ab-normal	In total	At normal	At ab-normal	Pro-thorax normal		No. of un-certain cases	Sum of columns 10 and 12	
1	2	3	4	5	6	7	8	9	10	11	12	13	14
BL. 162	1	50	50	—	1	109	94	15	21	3.4:0.5	2	23	3.1:0.8
" 163	2	32	31	1	—	70	58	12	4	3.3:0.7	10	14	3.2:0.8
" 164	3	34	34	—	3	122	96	26	24	3.1:0.8	11	35	2.8:1.1
" 164 Dp	4	39	37	2	2	90	68	22	19	3.0:1.0	6	25	2.9:1.1
" 165	5	53	52	1	—	23	13	10	*	2.2:1.7	*	*	—
" 167	6	50	50	—	—	4	3	1	2	3.0:1.0	—	2	2.0:2.0
" 170	7	30	*	*	—	27	19	8	5	2.8:1.2	4	9	2.6:1.3
" 173	8	15	15	—	5	29	21	8	*	2.9:1.1	*	*	—
" 166 Dp	9	34	32	2	1	80	60	20	16	3.0:0.9	6	22	2.9:1.1
" 168	10	50	49	1	—	53	44	9	9	3.3:0.7	—	9	3.3:0.7
Average	—	387	350	7	12	607	476	131	100	3.1:0.9	39	139	3.0:1.0

\* = not recorded.

In No. 1 to No. 8 inclusive, the ♂ was abnormal, the ♀ normal, No. 9 and No. 10 were reciprocals.

The average ratio between normal and abnormal antennae was as 3.1 : 0.9, consequently a monohybrid segregation.

Though the normal condition is dominant, we see that in the  $F_1$ , amongst the 357 beetles (from the 30 individuals of cross No. 7 no record was kept) seven had abnormal antennae, whilst of 334 beetles (the 53 beetles of cross No. 5 were not registered) 12 individuals showed a broad prothorax.

If the segregation of the broad prothorax is calculated for the average we find that for the 555 beetles (cross No. 5 and No. 8 being excluded) the average ratio is 3.3 normal : 0.7 abnormal. In most of the crosses the segregation of this character is less good than that of the abnormal antennae.

There are two reasons why the two characters do not always seem to go together:

1. The classification of the dimensions of the prothorax, as was already mentioned, is not based on actual measurements but on inspection, which is open to error; the more so as:

2. The variation in this organ makes it sometimes difficult to decide whether a specimen is normal or abnormal.

In such instances the beetles in the list were marked with a ?; the number of these uncertain cases is noted in column 12. When the figures of columns 10 and 12 are added together (column 13) and the proportion calculated, we may be content with the obtained ratio, the average of which is 3 normal : 1 abnormal.

#### *b. The strain At 10/10.*

In a culture started with the sole purpose of keeping in stock a homozygous race of yellow-eyed beetles (black being normal) a few males and females, eight in all, which showed that eye colour, were mated.

The appearance in the  $F_2$  of seven beetles with 10-jointed antennae and reduced tarsi awakened our interest to know how the  $P$  parents looked in that respect. It is our practice to preserve as far as possible the beetles used as parents in any experiment, and in this case, of the original eight parents, though two were lost, enough of the other six remained to prove that:

in one beetle both antennae were 10-jointed, and all six tarsi reduced with one segment;

in one beetle the six tarsi were also reduced, the antennae were broken off and lost;

in one beetle the legs were wanting, but the antennae, still entire, showed ten joints.

So at *least* three individuals of these eight parents were abnormal either in their antennae or in their tarsi, very likely all three in both organs.

Fifty  $F_1$  beetles *all* showed the normal number of tarsal joints; the antennae were not studied.

Of 110  $F_2$  individuals 99 were normal. In seven of the 11 abnormals *both* antennae and *all* tarsi had one joint less than the normal; the four others were abnormal either in the antennae or in one or more tarsi.

These seven beetles, having in both antennae and all tarsi *one* joint less than the normal, were mated for an  $F_3$   $A$ ; this was kept separate from the  $F_3$   $B$  resulting from the mating of the remaining 99 normal ones. These  $F_3$   $B$  gave 83 beetles which, with the exception of one individual, had all normal antennae and normal tarsi. The single abnormal beetle showed the composition At 10/10, T 4/4, 4/4, 4/3.  $F_3$   $B$  was not continued.

$F_3$   $A$  yielded 20, all abnormal, either in the antennae or in the tarsi or in both (columns 5 and 6, Table IV). They were all kept together to breed  $F_4$ , which contained 126 individuals, 123 or 98 per cent. being abnormal (columns 5 and 6). From these  $F_4$  beetles three groups were raised in  $F_5$  as follows:

$F_5$   $A$ , resulting from 15  $F_4$  beetles (column 13) in which the antennae were abnormal, showing ten joints, but all the tarsi normal, so At 10/10, T 5/5, 5/5, 4/4.

$F_5$   $B$ , resulting from two (one ♂ and one ♀)  $F_4$  beetles (column 8) with normal antennae but with all tarsi having one joint too few, so At 11/11, T 4/4, 4/4, 3/3.

$F_5$   $C$ , resulting from 14  $F_4$  beetles (column 15), all with reduced antennae and tarsi, so At 10/10, T 4/4, 4/4, 3/3.

$F_5$   $A$  gave 35 beetles,  $F_5$   $B$  18,  $F_5$   $C$  41, in total 94 beetles. This selection had no effect, all three groups producing similar combinations in antennae and tarsi.

We, therefore, did not continue this selection and, for simplicity's sake, gave no separate account of them in the Table IV, but counted them together as 94  $F_5$  beetles from which the  $F_6$   $A$  is obtained. In  $F_6$   $A$  there were 74 beetles, of which 32 (column 15) or 43 per cent. had in antennae and all six tarsi one joint less. Only beetles having that composition were kept for the production of  $F_7$   $A$  in which the same selection, with a view to  $F_8$  was applied.

TABLE IV.

*The strain At 10/10.*

From the total abnormals (column 5) the following cases were observed																
Beetles obtained						A		B				C				
Reference	Genera- tion	in total	nor- mal	Abnormal		Both antennae normal. Tarsus		One or both antennae semi-normal. Tarsus				Both antennae reduced to 10 joints. Tarsus				
				no.	in %	one or more reduced	all 6 reduced	all 6 norm.	one or more reduced	all 6 reduced		all 6 norm.	one or more reduced	all 6 reduced		
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
Kr.	21	F <sub>3</sub> A	20	—	20	100	—	2	—	12	1	5	—	5	0	
"	21	F <sub>4</sub>	126	3	123	98	5	2	8	23	15	12	15	41	11	
"	21	F <sub>5</sub>	94	—	94	100	1	5	2	12	14	15	3	27	31	
"	21	F <sub>6</sub> A	74	—	74	100	—	—	2	3	22	30	2	13	32	
BL.	182	F <sub>6</sub> B	83	—	83	100	2	1	—	18	22	26	—	13	43	
Kr.	21	F <sub>7</sub> A	59	1	58	98	1	—	1	2	5	8	—	6	73	
BL.	182	F <sub>7</sub> B	69	—	69	100	—	—	—	1	3	4	—	4	88	
Kr.	21	F <sub>8</sub>	36	—	36	100	—	—	—	—	9	25	—	9	50	
Total and average 561						9	10	13	71	91	16	20	118	225	40	
557																
D																
Summary with respect to the antennae alone (disregarding the tarsi)																
Reference	Genera- tion	Norm. (4, 7, 8)	One or both semi- norm. (9, 10, 11)	Both antennae abnormal (10 joints) (13, 14, 15)		Abnormal (18, 19)		Normal (4, 8, 13)	One or more reduced (7, 10, 14)	All 6 reduced (8, 11, 15)	Abnormal (24, 25)		in total	in %	in total	in %
				no.	in %	in total	in %				in total	in %				
Kr.	21	F <sub>3</sub> A	17	18	19	20	21	22	23	24	25	26	27	28	29	30
"	21	F <sub>4</sub>	10	46	70	56	116	92	26	69	31	25	100	79	93	74
"	21	F <sub>5</sub>	6	28	60	64	88	94	5	40	49	52	89	95	83	88
"	21	F <sub>6</sub> A	—	27	47	63	74	100	4	16	54	73	70	95	70	95
BL.	182	F <sub>6</sub> B	3	40	40	48	80	96	—	33	50	60	83	100	80	96
Kr.	21	F <sub>7</sub> A	2	8	49	83	57	97	2	9	48	81	57	97	56	95
BL.	182	F <sub>7</sub> B	—	4	65	94	69	100	—	5	64	93	69	100	69	100
Kr.	21	F <sub>8</sub>	—	9	27	75	36	100	—	9	27	75	36	100	36	100
Total and average		23	175	363	65	538	96	37	198	326	58	524	93	505	90	
561																
561																
E																
Summary with respect to the tarsi alone (disregarding the antennae)																
Reference	Genera- tion	Norm. (4, 7, 8)	One or both semi- norm. (9, 10, 11)	Both antennae abnormal (10 joints) (13, 14, 15)		Abnormal (18, 19)		Normal (4, 8, 13)	One or more reduced (7, 10, 14)	All 6 reduced (8, 11, 15)	Abnormal (24, 25)		in total	in %	in total	in %
				no.	in %	in total	in %				in total	in %				
Kr.	21	F <sub>3</sub> A	17	18	19	20	21	22	23	24	25	26	27	28	29	30
"	21	F <sub>4</sub>	10	46	70	56	116	92	26	69	31	25	100	79	93	74
"	21	F <sub>5</sub>	6	28	60	64	88	94	5	40	49	52	89	95	83	88
"	21	F <sub>6</sub> A	—	27	47	63	74	100	4	16	54	73	70	95	70	95
BL.	182	F <sub>6</sub> B	3	40	40	48	80	96	—	33	50	60	83	100	80	96
Kr.	21	F <sub>7</sub> A	2	8	49	83	57	97	2	9	48	81	57	97	56	95
BL.	182	F <sub>7</sub> B	—	4	65	94	69	100	—	5	64	93	69	100	69	100
Kr.	21	F <sub>8</sub>	—	9	27	75	36	100	—	9	27	75	36	100	36	100
Total and average		23	175	363	65	538	96	37	198	326	58	524	93	505	90	
561																
561																
F																
Summary, one or both antennae abnorm. simulta- neously with one or more abnorm. tarsus (10, 11, 14, 15)																
Reference	Genera- tion	Norm. (4, 7, 8)	One or both semi- norm. (9, 10, 11)	Both antennae abnormal (10 joints) (13, 14, 15)		Abnormal (18, 19)		Normal (4, 8, 13)	One or more reduced (7, 10, 14)	All 6 reduced (8, 11, 15)	Abnormal (24, 25)		in total	in %	in total	in %
				no.	in %	in total	in %				in total	in %				
Kr.	21	F <sub>3</sub> A	17	18	19	20	21	22	23	24	25	26	27	28	29	30
"	21	F <sub>4</sub>	10	46	70	56	116	92	26	69	31	25	100	79	93	74
"	21	F <sub>5</sub>	6	28	60	64	88	94	5	40	49	52	89	95	83	88
"	21	F <sub>6</sub> A	—	27	47	63	74	100	4	16	54	73	70	95	70	95
BL.	182	F <sub>6</sub> B	3	40	40	48	80	96	—	33	50	60	83	100	80	96
Kr.	21	F <sub>7</sub> A	2	8	49	83	57	97	2	9	48	81	57	97	56	95
BL.	182	F <sub>7</sub> B	—	4	65	94	69	100	—	5	64	93	69	100	69	100
Kr.	21	F <sub>8</sub>	—	9	27	75	36	100	—	9	27	75	36	100	36	100
Total and average		23	175	363	65	538	96	37	198	326	58	524	93	505	90	
561																
561																

In  $F_7 A$  73 per cent. of the individuals showed At 10/10, T 4/4, 4/4, 3/3 (column 16). In  $F_8$  the proportion fell back to 50 per cent. (column 16). If, however, we add the percentages given in column 12 (the antennae being partially fused) and column 16 (the antennae being completely fused) we find

$$F_6 A \quad 30 + 43 = 73 \text{ per cent. abnormals in 74 beetles}$$

$$F_7 A \quad 8 + 73 = 81 \quad , \quad , \quad 59 \quad ,$$

$$F_8 \quad 25 + 50 = 75 \quad , \quad , \quad 36 \quad ,$$

## 22      *Variation in the Mealworm, Tenebrio molitor*

i.e. the relative number of individuals showing a reduction in all six tarsi coinciding with a partial or whole fusion of the antennal joints is in these three generations nearly equal.

In comparing these figures, and their average, 75 per cent., with those (columns 12 and 16) for  $F_3$  with 5 per cent.,  $F_4$  with 23 per cent. and  $F_5$  with 46 per cent., we notice that the percentage of abnormals (having a reduction in all six tarsi and a partial or complete reduction in one or both antennae) has been increased from 5 per cent. in  $F_3$  to 73 per cent. in  $F_6$ , remaining nearly stationary in  $F_7$  (81 per cent.) and  $F_8$  (75 per cent.).

For reasons which had nothing to do with the present subject we had chosen out of  $F_5$  one pair of beetles from which the 83  $F_6$   $B$  and 69  $F_7$   $B$  individuals are the descendants.

That single  $F_5$  pair showed the following composition:

♂: At semi 10/semi 10, T 4/4, 4/4, 3/4.

♀: At semi 10/semi 10, T 5/5, 5/5, 4/4 (also nearly normal).

From the 83  $F_6$   $B$  beetles, 49 individuals or 58 per cent. (columns 12 + 16) had a reduction of one joint in all six tarsi, whilst the antennae were partially (26 per cent.), or wholly (32 per cent.) reduced.

In  $F_6$   $B$  we selected for  $F_7$   $B$ , only those beetles in which *all* tarsi and both antennae were reduced by one joint, viz.

At 10/10, T 4/4, 4/4, 3/3 individuals.

Here also selection gave a positive result, for from the 69  $F_7$   $B$  beetles 64, or 92 per cent., showed a reduction in all six tarsi, of which 4 per cent. had a partial (column 12) and 88 per cent. (column 16) a complete reduction to ten joints in the antennae.

For the moment we will postpone discussion of these facts and proceed with the explanation of Table IV. The different cases presenting themselves are grouped under three heads,  $A$ ,  $B$  and  $C$ , according to the condition of the antennae.

In  $A$  all the beetles had the normal number, 11 joints, in both antennae.

In  $C$  both antennae were reduced to ten joints.

In  $B$ , the semi-normals, the following cases occurred:

1. One antenna perfectly normal, in the other 4th and 5th joints partially fused.

2. One antenna normal, the other consisting of ten joints without a trace of fusion of 4th and 5th joints.

3. In both antennae 4th and 5th joints are partially fused.

4. One antenna with ten joints, in the other 4th and 5th partially fused.

These four cases may be regarded as intergradational steps from one extreme (both antennae normal) to the other extreme (both antennae having ten joints). The heads *D*, *E* and *F* give a summary, in *D* regarding the antennae only, in *E* regarding the tarsi only, in *F* in respect of a simultaneous reduction of antennae and tarsi.

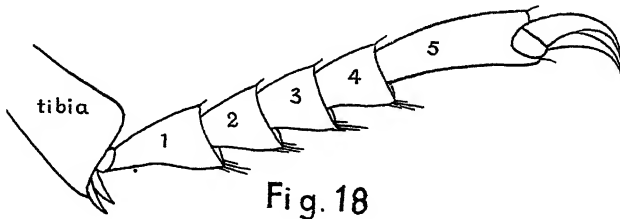


Fig. 18

I have given all these details, not because I attach particular significance to them but merely to show how extraordinarily variable the arrangements are. They may be summarised in the conclusion that, if one or more genetical factors are responsible, they manifest themselves in a tendency to diminish the normal number of joints in antennae and tarsi by one segment, and this may occur in only one tarsus of the six, or in only one antenna, or in all tarsi and both antennae.

We see that from the 561 investigated beetles only four were normal, 99.3 per cent. were abnormal (column 6, Table IV).

An analogous series can be made from the conditions of the tarsi, but the possibilities which may present themselves are so much greater, for the organs concerned are six instead of two.

On observing an extended normal tarsus (Fig. 18 of the 2nd leg) from the dorsal side, the following facts may be stated:

The 2nd and 3rd and 4th joints are of equal length; each of them is shorter than the 1st or 5th joint, which last two are respectively about  $1\frac{1}{2} \times$  and about  $2\frac{1}{2} \times$  the length of any of the middle joints (Fig. 18).

When reductions are observed, they occur, for the first and second pairs of legs, by a fusion of the 2nd and 3rd joints; for the third pair of legs by a fusion of the 1st and 2nd joints. In such a laterally extended leg we may distinguish an anterior and a posterior border of the tarsus as a whole.

At the posterior border of the tarsus each joint (except the last) bears on its distal angle two or more stiff chitinous hairs.



Also at the anterior border of the tarsus each joint bears at its distal angle a stiff chitinous hair; also the distal edge of each joint is set with a corona of fine hairs; they are, however, small and only fully observable by using a stronger lens ( $10\times$ ). For simplicity's sake we neglect them here, fixing our attention only on the more conspicuous double hairs at the posterior border of each tarsus.

When a tarsus has a supernumerary joint, that new joint shows also the above-mentioned double hairs.

When, however, the tarsus is reduced by one joint, different transitional conditions between the normal and abnormal can be noticed, of which the following, as being the most clearly differentiated may be mentioned, taking as example the second leg.

1. The 2nd and 3rd joints partially fused; a suture is visible; the fused joint being longer than the next, bearing a double set of chitinous hairs, which, however, have approached each other.

2. The 2nd and 3rd joints almost wholly fused; a *trace* of a suture still visible; the fused joint a trifle longer than the next; a less-developed accessory, often single, chitinous hair originating at the suture may be detected.

3. The 2nd and 3rd joints completely fused; no trace of a suture; the length of the fused joint equals that of the next joint or it is scarcely noticeable any longer; no accessory hairs.

These three types are examples selected from cases in which variations in the chitinous hairs, often fused to one bundle, or forming a continued row of hairs, may be noticed.

The difference in conditions described for types 2 and 3 were studied but recently with a stronger lens ( $10\times$ ) than we used to work with ( $6\times$ ).

Amongst the reductions occurring in all six tarsi mentioned in the columns 6, 11 and 15 of Table IV, several will therefore, by a more minute investigation ( $10\times$ ), answer to the type 2, making, however, no fundamental difference in the facts observed.

It remains still to be mentioned that also in this strain (At 10/10) the rounding of the front-horns of the prothorax and its reduction in length (mentioned for the strain At c) was often met with, though *not* so generally and so strikingly as in the former strain. A reduction in the length of the abdomen was also, not seldom, noticed, reaching the condition figured in my former paper in [(1a) Fig. 12 b, p. 257] in which both elytra overlapped the abdomen distally in a conspicuous manner.

Though the phaenotypical appearances of the strains At c and At 10/10 are, for anyone familiar with both types, quite different, it may

be that, on account of the similar deviations in antennae and prothorax and also in the reduction of the tarsi (though more as an exception in At c), the factors underlying these anomalies are for both strains the same. Though I do not think that this is the case, I have till now not been able to prove it, for reasons which are mentioned in the last chapter.

We made 19 crosses of individuals from the strain At 10/10 with normal beetles. In crosses No. 1 to No. 8 (inclusive), the ♂ was normal; in No. 9 to No. 19 inclusive (the reciprocals) the ♀ was normal. In the cross No. 8 the ♂, in No. 17 the ♀ belonged to the strain T a (see p. 27) and both can be considered as normal with respect to the other parent belonging to At 10/10. The results of these crosses, as well as the composition of the antennae and tarsi of the abnormal *P* parents, are tabulated in Table V.

The abnormal beetles obtained in  $F_2$  were, according to the condition of the antennae and tarsi, grouped in three sections. Column 7 contains the number of beetles with normal antennae but with one, more, or all six tarsi reduced; the sum of the columns 8 + 9 gives the number of individuals with reduced antennae (10 or semi 10); in column 8 when with the reduced antennae the tarsus was normal; in column 9 when together with the antennae one or more or all six tarsi were reduced.

The segregation is that of a monohybrid cross. Not only in their general average of 2.9 normals : 1.1 abnormals, but also in the crosses Nos. 1, 9, 10, 11, 12, 15 and 19, taken each by itself, this segregation comes so near to the theoretical ratio of 3 : 1 that any doubt about the meaning of these figures may be set aside.

In columns 3 and 4 we have given the number of beetles obtained in  $F_1$ ; in column 3 the totals, in column 4 the exceptionally-appearing abnormals.

In seven of these 19 crosses all the 140  $F_1$  beetles were normal; of the remaining 12 crosses from No. 19 no record was kept; in the other 11 crosses, giving in total 362 beetles, 24 abnormals appeared.

On pp. 2 and 8 it was stated that on investigating a population of beetles at random, reductions in antennae or tarsi which appear only in one organ, for instance, in one antenna, or in one of the six tarsi, may not *a priori* be considered as a fortuitous modification. In the two strains treated (At c and At 10/10) we have the actual proof of that assertion. Most of the exceptionally-appearing  $F_1$  beetles, mentioned in column 4, Table V, show such partial reductions, either in one antenna or in one tarsus. They are also found in the pure strain, but only as rare exceptions (Table IV, columns 7 and 9, from  $F_6 A$  to  $F_8$  inclusive). Such

TABLE V.  
The strain At 10/10. ♂ normal × ♀ abnormal.  
Beetles obtained in  $F_2$ .

Reference	No. of cross	Beetles obtained in $F_1$		In antennae		In total		Normal Tarsus reduced in total		Antennae		Reduced		In total Tarsus antennae reduced or tarsi		$F_2$ ratio of normal: abnormal		Composition of the abnormal parent	
		In total	With ab-normal	In antennae	In total	Normal	Tarsus reduced	In total	Tarsus reduced	Antennae		Reduced		In total Tarsus antennae reduced or tarsi		$F_2$ ratio of normal: abnormal		Composition of the abnormal parent	
1	2	3	4	5	6	7	8	9	10	Antennae		Reduced		In total Tarsus antennae reduced or tarsi		$F_2$ ratio of normal: abnormal		Composition of the abnormal parent	
BL. 131 C	1	18	1	62	46	4	3	9	16									14	
" 131 C.Dp	2	32	3	146	86	13	5	42	60									At 10/10	
" 152	3	27	1	67	46	4	1	16	21									"	
" 152 Dp	4	8	—	71	51	4	3	13	20									"	
" 183	5	4	—	34	23	1	1	9	11									"	
" 184	6	26	1	97	77	1	3	16	20									"	
" 188	7	40	—	106	89	—	11	6	17									At semi 10/10	
" 203	8	16	1	127	82	13	14	18	45									At semi 10/semi 10	
Total and av.	—	171	7	710	500	40	41	129	210							2:8:1:2			
BL. 96	9	18	1	73	55	—	14	4	18									The ♂	
" 96	10	22	—	67	48	—	18	1	19									At 10/10	
" 189	11	34	—	107	78	2	10	17	29									"	
" 190	12	33	1	97	70	3	6	18	27									"	
" 191	13	25	—	138	96	5	4	33	42									At 11/11	
" 192	14	68	4	75	62	1	5	7	13									At 10/10	
" 193	15	40	8	205	151	4	15	35	54									"	
" 194	16	34	1	55	44	—	5	6	11									At 11/10	
" 207	17	7	—	93	67	6	7	13	26									At 10/10	
" 147 Dp	18	50	2	150	120	3	6	21	30									At 10/10	
Kr. 30	19	42	*	74	56	—	12	6	18									"	
Total and av.	—	373	17	1134	847	24	102	161	287							3:0:1:0			
General total and average	—	544	24	1844	1347	64	143	290	497							2:9:1:1			

\* = not recorded.

cases are to be considered as extreme variants of a race which in their other extreme show those reductions in both antennae and all six tarsi.

*c. The strain T a.*

In a cross of one ♂ and one ♀ being mated with the intention to study a particular feature in the form of the prothorax, there appeared among the 62  $F_2$  beetles obtained, seven individuals which showed a strange deformity in all the six tarsi. The 92  $F_1$  beetles of this cross were all normal, also the 50 individuals obtained in  $F_3$  bred from normal  $F_2$  beetles. In  $F_4$ , however, amongst 31 beetles three showed the anomaly again.

$F_5$ ,  $F_6$  and  $F_7$ , giving 26, 50 and 57 beetles, were normal. From  $F_1$  to  $F_7$  inclusive, 368 beetles in all, the anomaly appeared thus in ten individuals. When the first beetle appeared we did not pay much attention to this deviation, but its repeated appearance and its symmetrical distribution in all six tarsi indicated that we had to do here with a genovariation and not with an accidental modification. The supposition proved to be true; the seven beetles mated produced an offspring in which *all* individuals exhibited the anomaly. It bred true during the five generations we kept; all of these 195 beetles without any exception showed the new character. Though the anomaly is manifested in all six tarsi, the condition is not in every tarsus the same. In fact, amongst a collection of these beetles, hardly two tarsi may be found exactly alike. In the former strain At 10/10 there was only question of a reduction in the *number*, the form of the tarsal joints remaining unaltered. When applying the same term (reduction) to this new strain, we should certainly give a wrong idea of the condition.

If we compare the joints of the normal tarsus with the joints of an extended telescope, we can slide *out* or push *in* the joints in different ways and combinations. We can collapse the 1st joint into the 2nd, or only the 2nd into the 3rd, or the 1st and 2nd into the 3rd, etc.

The anomaly in this strain may be compared with the different possibilities in which the telescope is partially drawn out. The disappearance of the joint, pushed in, is often so complete that not a trace of it is discernible (Fig. 19 *a* 2; 19 *b* 1 and 2; 19 *f* 1). Not seldom may an abnormal thickening and enlargement of the remaining joints be observed, forming an irregular shaped body often provided with excrescences, tubercles or spine-like processes (Fig. 19 *d* 1 and 3; 19 *e* 1 and 2). The five or four members of the tarsi may disappear so completely that only a small rudiment of them is detectible (Fig. 19 *f* 3 and 4), the two claws of the leg are then seen on the end of the tibia.

As I stated already, the conditions found in a population of this race are so widely different that scarcely two tarsi may be found alike.

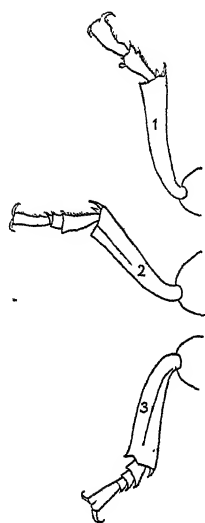


Fig. 19a

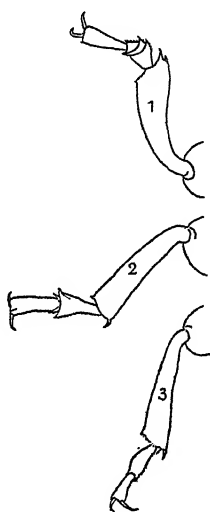


Fig. 19b

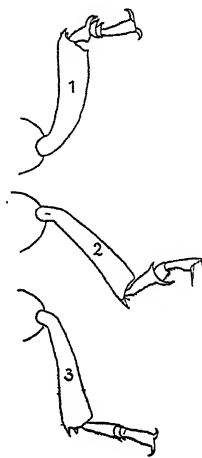


Fig. 19c

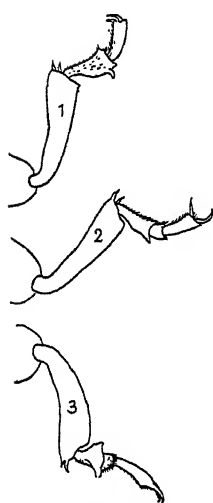


Fig. 19d

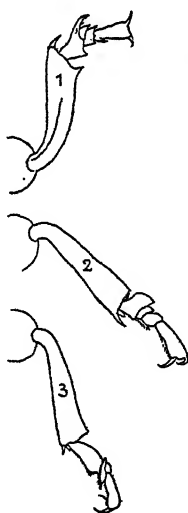


Fig. 19e

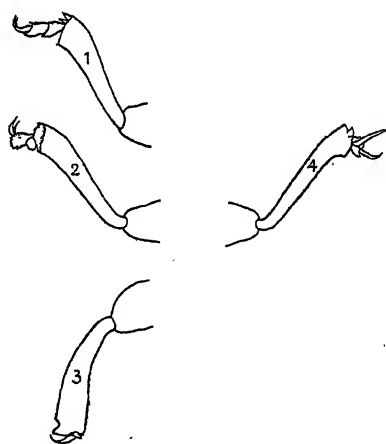


Fig. 19f

On account of the unstable abnormal condition of the tarsus by which this race is characterised, we will henceforth denote it by T a (Tarsus

abnormal). The antennae in this race T a were perfectly normal, not a trace of fusion or reduction of its joints was ever noticed.

The same can be said of the dimensions of the prothorax and the form of its front horns, which exhibited the normal condition.

Crosses of this strain with normal beetles gave in the  $F_2$  a monohybrid segregation as shown in Table VI. The normal condition is dominant; the tarsi of the 402 investigated  $F_1$  beetles of these 15 crosses were all normal:

TABLE VI.  
*The strain T a. ♂ normal × ♀ T a.*

Reference	No. of cross	Beetles obtained in $F_2$			Ratio pro. 4 abnormal : normal
		Total	Normal	With T a	
BL. 66	1	48	36	12	1.0 : 3.0
" 88	2	36	27	9	1.0 : 3.0
" 92	3	120	97	23	0.7 : 3.2
" 158	4	61	50	11	0.7 : 3.2
" 159	5	7	5	2	1.1 : 2.8
" 170	6	27	20	7	1.0 : 2.9
" 173	7	29	22	7	1.0 : 3.0
" 108	8	67	54	13	0.8 : 3.2
" 202	9	93	67	26	1.1 : 2.9
Total and average	—	488	378	110	0.9 : 3.1
<i>♂ T a × ♀ normal.</i>					
BL. 155	10	54	48	6	0.4 : 3.5
" 155 Dp	11	72	59	13	0.7 : 3.2
" 156	12	24	20	4	0.6 : 3.3
" 105	13	60	46	14	0.9 : 3.0
" 157	14	84	67	17	0.8 : 3.1
" 203	15	127	94	33	1.0 : 3.0
Total	—	421	334	87	0.8 : 3.1
Total of both groups	—	909	712	197	0.8 : 3.1

*d. Crosses of T a × At 10/10.*

Two crosses were effected between the strains At 10/10 and T a. Their results are given in the following survey:

Reference	No. of cross	$F_2$				
		Total	At normal T normal	At 10/10 T normal	At normal T a	At 10/10 T a
BL. 202	1	93	48	19	19	7
		Pro. 16	8.2	3.3	3.3	1.2
Expectation	—	Pro. 16	$9 \pm 0.8$	$3 \pm 0.6$	$3 \pm 0.6$	$1 \pm 0.4$
Deviation	—	—	-0.8	+0.3	+0.3	+0.2
BL. 203	2	127	66	28	24	9
		Pro. 16	8.3	3.6	3.0	1.1
Expectation	—	Pro. 16	$9 \pm 0.7$	$3 \pm 0.5$	$3 \pm 0.5$	$1 \pm 0.3$
Deviation	—	—	-0.7	+0.6	0	+0.1
Total and average	—	220	114	47	43	16
		Pro. 16	8.3	3.4	3.1	1.2
Expectation	—	Pro. 16	$9 \pm 0.5$	$3 \pm 0.4$	$3 \pm 0.4$	$1 \pm 0.2$
Deviation	—	—	-0.7	+0.4	+0.1	+0.2

30 *Variation in the Mealworm, Tenebrio molitor*

On account of the fact already mentioned that the antennae of the T a strain are perfectly normal, we never felt any hesitation, in  $F_2$ , regarding the classification of that organ.

In the tarsi, however, sometimes cases were met with in which it was not *always* directly clear, if such a tarsus belonged to a T a, or to an At 10/10 strain. The fact so often seen, that the six tarsi of a T a beetle scarcely ever show precisely the same aspect, taken as a guide, always helped us, in such a case, out of the difficulty.

*e. Crosses of T a  $\times$  At c.*

Also between the strains At c and T a two crosses were started. The results given below correspond only partially to those of a dihybrid cross.

*Crosses of At c  $\times$  T a.*

Reference	No. of cross	$F_2$				
		Total	At normal T normal	At c T normal	At normal T a	At c T a
BL. 170	1	27	17	3	2	5
		Pro. 16	10	1.8	1.2	3
Expectation	—	Pro. 16	$9 \pm 1.5$	$3 \pm 1.2$	$3 \pm 1.2$	$1 \pm 0.7$
Deviation	—	—	+1	-1.2	-1.8	+2
BL. 173	2	29	18	4	3	4
		Pro. 16	9.9	2.2	1.7	2.2
Expectation	—	Pro. 16	$9 \pm 1.5$	$3 \pm 1.2$	$3 \pm 1.2$	$1 \pm 0.7$
Deviation	—	—	+0.9	-0.8	-1.3	+1.2
Crosses 1 and 2	—	56	35	7	5	9
		Pro. 16	10	2	1.4	2.6
Expectation	—	Pro. 16	$9 \pm 1.1$	$3 \pm 0.8$	$3 \pm 0.8$	$1 \pm 0.5$
Deviation	—	—	+1	-1	-1.6	+1.6

There is an excess of the recessive combinations. If this excess is fortuitous, and due to the small number, the fact should appear when the result of more extended experiments is obtained. On another occasion we will return to this point again.

As regards crosses between At c and At 10/10 special difficulties of determination were met with, which we still hope to overcome.

## D. DISCUSSION.

Are the variations described in this paper meristic? To answer this we have to ascertain first what by this term has to be understood and what it includes. On this point any obscurity must, if possible, be cleared up, because the conception as to what a meristic variation *is*, and what it *is not* will lead, for a given case, to important consequences.

A character is spoken of as meristic "when it manifests itself in respect of the number of parts into which the body or one of its organs is divided" [(3), p. 47]; or

"Variations in the processes of division are most often made apparent by a change in the number of the parts, and are therefore called *Meristic Variations*" [(4), p. 31].

According to these (Bateson's) definitions, the variations here treated above show, taken in a general sense, a meristic feature, because they all relate to a change in the number of divisions of segmented organs.

The definitions, however, involve more, they want the further elucidation that:

"Meristic Variation is frequently Discontinuous and in the case of certain classes of Repetitions is perhaps always so" [(2), p. 23].

What does Discontinuity mean here?

Does it mean that between the  $n$ -jointed and  $n-1$ -jointed conditions there is no intermediate condition as in the case of *Blatta* mentioned by Bateson [(2), pp. 63 and 417]? Or may it be taken in the sense that though many transitional cases are observed, they have not to be gone through as intercalated steps in the line of descent from the normal to the perfect abnormal condition [(2), pp. 62 and 65]? The deviation in our strain At 10/10 for instance, though showing nearly all the possible intermediate gradations connecting by a long continuous chain the normal with the perfect abnormal, is, with respect to the normal type, of course discontinuous. There remains still a gap between the normal and abnormal type, but this gap is so completely overbridged that we scarcely can find out the exact point where the bridge commences.

But this conception of discontinuity is perhaps not meant here. Is not the meaning rather that *within* the strain itself gradational steps are *totally absent*? I do not think it is; both cases are possible, viz.: *with* and *without* intermediates. Bateson is quite clear upon this point:

"It is not intended to affirm that in discontinuous Variation there can be between the variety and the type no intermediate form, or that none has been known to occur, and it is not even necessary for the establishment of Discontinuity that the intermediate forms should be rare relatively to the perfect form of the variety, though in cases of discontinuous Variation this is generally the case; but it is rather meant that the perfect form of the variety *may* appear at one integral step in Descent, either without the occurrence of intermediate gradations, or at least without the intercalation of such graduated forms in the pedigree" [(2), p. 66].

So the meaning is that in meristic variations intermediate forms are as a rule wanting; they may, however, occur.



So we may conclude that the many transitional conditions found in our strains At c and At 10/10 are no fundamental objection for conceiving these variations as truly meristic.

It may, however, be asked whether the *manner* in which such variations came into being has *also* to be taken into account before a given case can be considered to be meristic. We know, for instance, that the reductions in our At c and At 10/10 strains arose by fusion of joints. If it had been otherwise, if the reduction had arisen by the *disappearance* of one joint (with or without intermediate conditions), would both cases have the same value regarded as meristic phenomena? I do not think they would.

In case of fusion there is *de facto* no reduction in the number, because in ontogenesis this number is normal; the fusion follows later on, as a secondary process. Such a fusion may, however, transform the adult antennae and tarsi so thoroughly, that no longer the slightest indication of a previous separate condition is detectable, neither by a *trace* of a suture, nor by the vestige of a constriction, nor by an abnormal length of the new-born joint. And these cases do not appear as exceptional conditions but are often noticed in many beetles. If meristic variation is conceived as a purely mechanical process of division, the reductions pictured in Fig. 1 to Fig. 11 inclusive, and those observed in our strains At c and At 10/10 may not be considered as true meristic variations.

So not every variation in *number* is meristic, because this word implies some preconceived conception as to the way in which such variation must or must not manifest itself, before the term "meristic" can be applied to it.

The 12-jointed antennae (see p. 5) and the cases of an increased number of tarsal joints (see Table I), however, fulfil in all respects the requirements of true meristic examples.

For the strain T a we are in doubt. An actual fusion of members has never been noticed. There is a tendency for one or more joints to fall out of the series. The tarsus may show four, or three, or two, or no joints at all, *not* by fusion, but by disappearance, and this disappearance may show different intermediate conditions as regards the size of the joints affected, from nearly normal to an absolute loss.

The facts of segregation of characters suggest that the genes, whose mutual interaction is made responsible for the manifestation or suppression of these characters, are represented in the cell by material bearers. Further, that the possession or the want of such a bearer will, in many cases, manifest itself in a difference of the phaenotypes attending that presence or absence.

It will be granted that the material conception of the nature of genes may be maintained for any sort of substantive character. Likewise there is not much to oppose against Bateson's view that "hardly by any effort of imagination" [(4), p. 35] can we maintain such an idea of genes as material bearers for characters which are meristic, that is, which are expressed in *numbers*.

This and other distinctions between the nature of substantive and meristic characters is by Bateson conceived as fundamental, they "must surely be of a different order" [(4), p. 35], and I think we cannot avoid coming to the same conclusion.

Yet the possibility must be accepted that change in the genes, i.e. change in the *quality* of the living matter itself (substantive), may interfere with a mechanical process of division in such a way that it inhibits the normal course of that process; the changed constitution of the material (substantive), may affect the *manner* in which that material is divided or distributed (meristic). The alterations in the quality of the material act then on the mechanical forces of division as a changed environmental condition. Indeed Bateson himself expressed such a view. Referring to the experiments of Loeb (the production of twins, i.e. abnormal *division*, in sea-urchin eggs temporarily immersed in lime-free sea-water), and of Stockard (the *suppression* of division, i.e. fusion, cyclopic monstrosities, in fish embryos treated with a dilute solution of magnesium salts), Bateson argues: "the facts suggest that these effects are due rather to alterations in the living material than to influence exerted directly on the forces of division" [(4), p. 71].

All this leads to the conclusion that meristic variation as "a divisional change, pure and simple...[not being] accompanied by change in the distribution of differentiation...[and] from which all confusing elements are eliminated must be rare" [(3), p. 47].

Taken in this most rigorous sense, none of the variations treated in this paper (with exception of those showing an *increase* in number) are meristic.

Their final result, diminution in number of the tarsal and antennal joints, is a concomitant phenomenon of a variation which is ultimately substantive in its nature. Looking from that standpoint at the obtained results with our three strains, we understand why they show the same instability in their phaenotypical appearance as such variations do which are strongly influenced by environmental agents. Between the two extremes (on one side the normal, on the other side the perfect abnormal) all intermediate transitions may be found. By a suitable classification,

and marshalling the classes in a definite order, it would be possible to construct from them a curve, which in its essential character would answer to the curve of error, just as if the variability of these strains is no more than the common fluctuating modifications which nearly every character of any organism is subjected to.

However, we have seen that it is more than that; that genetical factors lie at the base of it; and as the presence (or absence) of these factors does not affect the number of partitions of antennae and tarsi in a clearly discontinuous manner, there is some reason to accept the view that their influence upon the mechanical process of division is of a secondary nature, impeding that process only in its normal course.

As far as I know, no cases are mentioned in genetical literature, of true meristic variations showing a Mendelian inheritance. Even the brachydactylism in man seems, according to Drinkwater (cited by Bateson), to originate by a fusion of the middle and distal phalanx [(3), p. 47].

It is also worth noticing that in our experiments the three pseudo-meristic deviations showing a *decrease* in number, followed a heredity along Mendelian lines, whilst in the cases showing an *increase* in number (12-jointed antennae; supernumerary joints in the tarsi) not any indication could be detected of genetical factors playing a part in it.

I do not mean to offer these results as a proof that true meristic changes (as being a pure mechanical process) are never ruled by genetical factors; but the facts (1) that cases showing that interdependence are not known, (2) that the variations dealt with in this paper are far from supporting such a conception, both in connection with the above-mentioned words of Bateson, viz. the impossibility to realise in thought a such like relation [(4), p. 35], suggest that such a relation does not exist, and will therefore never be found.

Before finishing this paper I want to say a few words about recommending *Tenebrio* as a suitable object for genetical research. Simple in its needs for the sustainment of its life, content with very little space which makes it possible to keep and breed it in large quantities at a minimal expense, easy in the study of its phaenotypical appearance, it can be propagated, without risk of epidemic disasters, at any season, the whole year round, at a rate which within a wide range can be artificially regulated. There are not many animals which can rival it with such good qualities for experimental study. The circumstance that one year is required before the  $F_2$  can be studied may in some cases be a serious objection, in others, however, be regarded as a great advantage, as it

allows the student, especially when only the solution of one or a few questions is aimed at, to work with it in moments of leisure which even any sphere of activity provides.

Besides *T. molitor*, we have also reared cultures of *T. obscurus* and *T. Syriacus* which are as easy to keep and propagate [(1 c), p. 142].

Besides the three strains, mentioned in this paper, there are some others relating to difference in larval colour, eye-colour of the beetles and deviations in the form of the prothorax.

For the sake of heightening the variability of my stock in order to obtain material for selection, several populations coming from different places in South and East Europe were mutually crossed. All this relatively rare material is far too much for one man to work out thoroughly, and I am quite willing to place a part of it at the disposal of students in genetical problems.

Applicants have to address themselves to the Director of the Laboratory of Embryology, Prof. Dr J. Boeke, at Utrecht, who had the kindness to offer me in his Institute the opportunity of keeping this material in stock.

## E. SUMMARY.

### MODIFICATIONS.

#### *In the antennae.*

1. *Reductions* in the number of the antennal joints generally manifest themselves in a partial or complete fusion of the two or more joints.

The fusion may reach such an extent that, with the exception of the scapus and pedicellus (which never have been observed in a fused condition), all the remaining nine members are represented by only one joint (Fig. 11).

2. An *increase* in the number of joints is rare; it was never observed that the increase amounts to more than *one* supernumerary joint, which arose by a partition of an abnormally enlarged joint.

Between the normal and the fully developed 12-jointed condition, transitional cases are observed (Fig. 1 a; 1 b; 1 c).

3. It is still uncertain whether the peculiar deviation in the antennae in which the terminal joint is Pixavon-shaped (Fig. 12) is a hereditary anomaly or only a variation of a personal character. As yet the selection has had no effect beyond that sometimes a large percentage (58) of the progeny of such selected beetles exhibits the anomaly in both antennae.

*In the tarsi.*

4. *Reductions* in the tarsi are, in contradistinction to the antennae, often observed without any indication that such reductions came about by a fusion of joints [(1a), p. 259, Figs. 13, 14, 15]. The tarsus may finally be reduced to one (terminal) joint [(1a), p. 259, Figs. 13 e; 14 e; 15 e].

5. An *increase* in the number of tarsal joints is far from common. We found 69 of such anomalies amongst the 42,487 investigated beetles.

In seven crosses (Table I) in which one of the parents was abnormal, the anomaly reappeared in nine individuals amongst the 570  $F_2$  beetles, imitating the ratio of a trihybrid cross, viz. one abnormal in 64.

This conception is not, however, supported by the other experiments mentioned.

## GENO-VARIATIONS.

6. Three geno-variations were obtained which we have denoted by the initials

At c (Antennae compressed).

At 10/10 (Antennae and tarsi reduced with one joint).

T a (Tarsi abnormal).

All three strains breed true, and all three show a monohybrid segregation when crossed with a normal individual.

7. *The strain At c* is characterised by the thick-set appearance of the body and their appendages (antennae and legs):

(a) Antennae, tarsi and prothorax are compressed and shortened in the axial line; these organs have lost their slender appearance; the joints are broadened; the front-horns rounded; the scutellum often pseudo-semicircular.

(b) The reductions either in antennae or tarsi are secondary; their number may be normal whilst these organs still show the characteristics specific to this strain.

(c) Complete fusions of two or more members give antennae in which ten, nine, eight or even seven joints may be counted. A fusion of the 4th and 5th joints occurs often, mostly accompanied with a partial or whole fusion of the more peripheral members.

(d) Reductions in the tarsi with one joint occur, but are far from general.

8. *The strain At 10/10* is characterised by a reduction in both antennae and all tarsi with one joint; the beetle showing then the composition of At 10/10, T 4/4, 4/4, 3/3.

(a) Antennae and tarsi keep their usual slenderness. Reductions in the length of the prothorax and a rounding of its front-horns occur, but are by no means so general as in the strain At c.

(b) The composition of the individual, mentioned above, may be observed in 88 per cent. of the beetles ( $F_2$ , B, columns 15 and 16, Table IV).

(c) Others may show reduced antennae with one or more reduced tarsi (column 14).

(d) Or, reduced antennae with normal tarsi (column 13).

(e) Or, semi 10/10 antennae with all tarsi reduced (columns 11 and 12).

(f) Or, semi 10/10 antennae with one or more tarsi reduced (column 10).

(g) Or, semi 10/10 antennae with normal tarsi (column 9).

(h) Or, normal antennae with all tarsi reduced (column 8).

(i) Or, normal antennae with one or more tarsi reduced (column 7).

The cases mentioned in b-i (inclusive), form a series of intergradational steps from the normal to the wholly abnormal condition.

9. The strain *Ta* is characterised by the antennae being always normal, whilst all six tarsi show abnormal conditions; this holds good for all the individuals obtained in this strain.

(a) The anomaly in the tarsi is highly variable; not only are the conditions in two beetles scarcely ever exactly alike, but the same may also be stated of the six tarsi in one individual, which, as a rule, all show a different aspect.

(b) The conditions observed may be compared to those obtained with a telescope tube in which the five members may be pushed in, or drawn out in different combinations, and by which *one* (Fig. 18 e, 2nd leg), or *two* (Fig. 18 c, 2nd leg) or *three* (Fig. 18 f, 1st leg) or *four*, or even all *five* joints may entirely disappear, in which last case the terminal claws originate from the tibia (Fig. 18 f, 3rd leg).

10. Crosses of the At 10/10  $\times$  *Ta* strain exhibit a clear dihybrid segregation.

11. The cross At c  $\times$  *Ta* gave an uncertain result.

In the two crosses which were made, the combination At c-*Ta* shows an excess over the two other possible combinations.

Whether this result is fortuitous, caused by the small number of the obtained  $F_2$  beetles (27 for cross No. 1, 29 for cross No. 2), or that certain combinations are more easily realised (linkage) than others, further experiments must reveal.

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# STUDIES ON THE SEX-RATIO AND RELATED PHENOMENA.

## II. THE INFLUENCE OF THE AGE OF THE MOTHER ON THE SEX-RATIO IN MAN.

By A. S. PARKES, B.A. (Cantab), Ph.D.,  
*University College, London.*

### CONTENTS.

	PAGE
Introductory . . . . .	39
The problem . . . . .	40
Objects . . . . .	41
The influence of the age of the mother . . . . .	42
The nature of the influence of the age of the mother and the parity . . . . .	42
The incidence of pre-natal mortality . . . . .	44
Conclusion . . . . .	45
Summary . . . . .	46

### INTRODUCTORY.

THE records which form the basis of the following investigation contain details of the patients admitted to the Obstetric Branch of St Mary's Hospital, Manchester, for the years 1911-1920.

The total number of patients for this period was 13,396, and of these 8384 have maternity details entered up. Of the remaining 5012, 1659 are abortions and the rest cases of puerperal complaints and non-deliveries.

Among the data the following are relevant to the problem in hand: age of mother, parity, sex and condition (live or dead) of births.

The age of premature births is only occasionally noted, and most unfortunately the age and sex of abortions are almost entirely omitted. The Hospital authorities gave me all assistance possible, and I should like to take this opportunity of expressing my very best thanks to Dr C. P. Brentnall, the Registrar and R.S.O., and also to his successor,



## 40 *Studies on the Sex-Ratio and Related Phenomena*

Dr von Mengershausen, for their great kindness and consideration in placing the records at my disposal and for allowing me the use of their room. I am indebted to Dr F. H. A. Marshall, F.R.S., of Christ's College, Cambridge, for his critical interest in the work.

### THE PROBLEM.

One of the gravest obstacles to the complete acceptance of the chromosome theory of sex determination in mammals, which postulates the male as the heterozygous and therefore sex determining parent, has been the acknowledged influence on the sex-ratio at birth of the age of the mother and the parity<sup>1</sup>. Goldschmidt (8), in particular, emphasises this discrepancy.

The apparently opposing sets of evidence may be briefly considered.

The evidence supporting the supposition that in mammals the male is heterozygous for sex is largely drawn from the manner of the inheritance of sex-limited characteristics and the cytology of spermatogenesis.

Guyer (9, 10) has described dimorphic spermatozoa in a negro, the spermatogonia having 22 chromosomes and the spermatozoa 10 or 12. Von Winiwarter (27), in examining the spermatogenesis of a white man, found 47 chromosomes in the spermatogonia and 23 or 24 in the spermatozoa. Paniter in a more recent paper (17) has described an *XY* hair in man, making the somatic number 48.

For other mammals a fair amount of data exist. Wodsedalek (29) found that in the bull the spermatogonia had 37 chromosomes, and the spermatozoa 18 or 19. The somatic number in the female was found to be 38, the ova all possessing 19 chromosomes. In the pig the same author (28) found that the spermatozoa had 8 or 10 chromosomes, while the eggs all had 10.

In view of Wodsedalek's work it is interesting that Wentworth (24) should have found a sex-limited characteristic in Ayrshire cattle which tends to show that the spermatozoa must be dimorphic.

Malone (15) found an unpaired heterochromosome in the spermatogenesis of the dog.

In the case of the rat Allen (2) has demonstrated an accessory chromosome in spermatogenesis, and the spermatozoa have 18 or 19 chromosomes. Von Winiwarter (26) has shown that the spermatozoa of the cat are cytologically dimorphic, one type having 18 chromosomes

<sup>1</sup> A convenient expression for the number of the pregnancy.

and the other 17, while Yocum (30) found the same thing to obtain in the mouse, where the spermatozoa have 19 or 20 chromosomes.

Lastly, the present writer (18) found that the male appeared to control the production of atypical sex-ratios in man.

There is thus a substantial body of evidence which seems to show that the mammalian male is heterozygous for the sex factor, and the female homozygous.

In contradiction to this there are the two factors relating to the maternal organism, the age of the mother and the parity, which undoubtedly influence the sex-ratio at birth.

It has been almost universally shown that the sex-ratio<sup>1</sup> of births declines with increasing age of the mother and at the higher parities, both of which factors relate to the female only.

Punnett (19) gives some most interesting tables on the sex-ratio of first, second and subsequent children. His statistics from Burke's peerage show a continuous drop in the ratio from the first child to the ninth. This is corroborated by his two tables compiled from the material collected by the late Dr Rivers in the Torres Straits. Similar results had previously been obtained by Düsing (6), by Ahlfeld (1) and Rosenfeld (21), and later by Newcomb (16) for man, whilst King (11) for rats, and Copeman and Parsons (5) for mice, have shown that the phenomenon is not confined to man.

There are two records of the sex-ratio increasing with parity which should be mentioned. Little (14) found by analysis of the records of Soloane Maternity Hospital, New York, that while in white subjects the sex-ratio decreased in later births, in coloured races just the opposite obtained, that the ratio of births of multiparae was higher than those of primiparae. Felkin also notices that among the natives of Africa the excess of female births is largely supplied by first births (7).

With regard to the influence of the age of the mother, Punnett (19) found that for first births the least excess of males occurred at the beginning and end of maturity of the mother. Punnett also analysed Düsing's statistics in the light of his own results and found that they were in agreement.

#### OBJECTS.

Confronted with these apparently opposing sets of facts the question arises as to whether they are really mutually contradictory, or whether

<sup>1</sup> Calculated as males per 100 females.

## 42 *Studies on the Sex-Ratio and Related Phenomena*

they can be reconciled. The objects of this paper are therefore to endeavour to throw some light on the nature of the influence of the age of the mother and the parity, and to find if any real contradiction to the chromosome theory is implied.

### THE INFLUENCE OF THE AGE OF THE MOTHER.

As the effect of the age of the mother has been less investigated than that of the parity, it seemed advisable to make some further investigation of this former question from the records of St Mary's Hospital. The births were analysed for different ages of the mother in groups of five years, and the results were as follows:

TABLE I.

*Age of Mother and Sex-Ratio. (St Mary's Hospital.)*

Age of Mother	Males	Females	Totals	Sex-Ratio
13—17 years	36	22	58	163·8
18—22 „	908	760	1668	119·8
23—27 „	1249	1129	2378	110·6
28—32 „	994	889	1883	111·7
33—37 „	716	635	1351	112·6
38—42 „	414	451	865	91·8
43+ „	83	98	181	84·6
Totals ...	4400	3984	8384	110·5

These figures show that the progeny of young and old mothers tends to have a high sex-ratio and a low sex-ratio respectively.

Taking into consideration the work mentioned above it may be said, therefore, that the sex-ratio declines with ageing of the mother and at the higher parities.

### THE NATURE OF THE INFLUENCE OF THE AGE OF THE MOTHER AND THE PARITY.

It should be emphasized immediately that the only sex-ratio which we know to be affected by either the age of the mother or the parity is the secondary sex-ratio, the proportions of the sexes at birth, while the facts of sex determination are concerned solely with the primary sex-ratio, the ratio at conception, or when sex is first determined. It is not impossible, therefore, that changes in the sex-ratio during gestation may account for the influence of factors relating to the maternal organism. If this were so the serious discrepancy between the two sets of facts would be cleared up. This possibility may be examined a little more closely.

After sex is determined changes in the proportions of the sexes can only come about, in the absence of complete sex reversal, by unequal elimination of the sexes, that is, by more of one sex dying than of the other. Thus if the amount of pre-natal mortality was greater in older mothers and at the higher parities than among younger mothers and earlier pregnancies, the observed result of a decreasing sex-ratio with advancing age of the mother and at the higher parities would be obtained, provided the amount of pre-natal mortality were appreciable, and fell most heavily on the males.

These three essentials may be considered separately. While pre-natal death, especially early in gestation, does not always end so, abortion is the only process which can be dealt with here.

The amount of abortion is undoubtedly considerable.

Routh (22) gives a table of collected calculations by various authorities where the estimates vary between 14 per cent. and 30 per cent. of all pregnancies. The figures average at about 20 per cent., which gives an abortion ratio of 25 per 100 births.

Whitridge Williams (25) estimates one abortion in five pregnancies, again an abortion ratio of 25. The St Mary's Hospital records show 1659 abortions to 8384 births, a ratio of 19.8, which agrees very closely with the figures given above. Thus there is no doubt about there being an amount of abortion adequate to affect the sex-ratio if unevenly distributed between the sexes.

With regard to this point, the sex incidence of abortion, there were no records at St Mary's Hospital, but fortunately there are several well authenticated investigations by various authors. The sex-ratio of abortions has variously been found to be 160.0 (Lenhossek, 13), 250 (Carvalho, 4), 152.4 (Körösy, 12), 159 (Rauber, 20), 156.4 (Auerbach 3), 118.7 (Schultz, 23). It would appear, then, that the sex-ratio of abortions may be confidently placed at about 150<sup>1</sup>.

It has thus been shown that the amount of abortion is considerable and falls more heavily on the males than on the females, and it now remains to consider from my own material the age of mother and parity incidence of pre-natal mortality.

<sup>1</sup> Günther in a recent paper (*Naturwiss. Korrespond.*, Jahrg. 1, 1923) has shown that both the amount of mortality and the sex-ratio of mortality probably follow a logarithmic curve back into pre-natal life. As there are no means of assessing the amount of mortality which occurs early in gestation, the wastage of males before birth is undoubtedly greater than the amount it is possible to compute.

# 44 *Studies on the Sex-Ratio and Related Phenomena*

## THE INCIDENCE OF PRE-NATAL MORTALITY.

Table II gives a full analysis of the figures yielded by the records of St Mary's Hospital.

TABLE II.

*Abortions according to Age of Mother and Parity with ratio to Births, and Number of Still-births with ratio to total Births.*

Age of Mother	Parity						Totals	Number of Births	Abortions per 100 Births	Number of Still-births	Number of Still-births per 100 total births
	1	2	3	4	5, 6 & 7	8+					
-17 years	1	—	—	—	—	—	1	58	1.7	0	0
-22 „	57	29	14	5	1	—	106	1668	6.3	145	8.7
-27 „	75	60	70	44	50	8	302	2378	12.7	285	12.0
-32 „	42	63	70	64	194	58	491	1883	26.1	316	16.8
-37 „	31	26	35	37	166	119	414	1351	29.9	309	22.9
-42 „	8	12	8	18	77	147	270	865	31.0	252	28.8
+ „	8	2	2	2	19	47	75	181	41.4	51	28.4
Totals	217	192	199	170	507	374	1659	8384	19.8	1358	16.2
Number of Births	3764	1334	843	589	1004	850	8384	—	—	—	—
Number of Abortions per 100 Births	5.7	14.4	23.6	28.9	48.8	44.0	19.8	—	—	—	—
Number of Still-births	500	168	142	106	201	241	1358	—	—	—	—
Number of Still-births per 100 total Births	13.3	12.6	14.2	18.0	20.0	28.1	16.2	—	—	—	—

This shows that the number of abortions rises steadily from 1.7 per 100 births in the case of mothers of from 13–17, to 41.4 in the case of mothers over 43 years of age. In the parity groups a similar result is observed, the number of abortions per 100 births rising with only one break from 5.7 for first pregnancies to 44.4 in the case of eight and higher pregnancies. For still-births<sup>1</sup> the like thing is found. In the age of mother groups the number per 100 total births rises almost continuously from 8.7 in the case of mothers of 18–22 years of age to 28.4 for mothers of 43 and more years of age. The number of still-births also increases in later pregnancies.

<sup>1</sup> Although still-births are not included in pre-natal mortality, it is obvious that they are roughly due to the same causes as abortions and are therefore comparable with them. If still-births were mainly due to labour troubles, which are most severe at first births, they would mostly occur early on. As, however, this is not so, still-births cannot mostly be due to labour troubles and must therefore be largely dead before parturition sets in.

These results may be represented graphically.

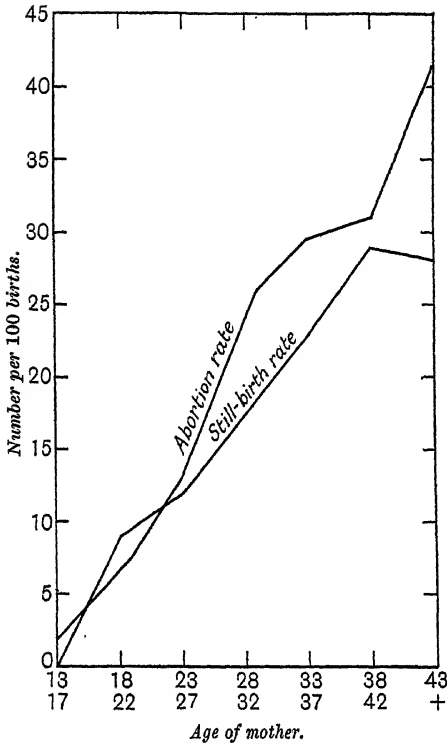


Fig. 1. Abortion and Still-birth rates at age of mother groups.

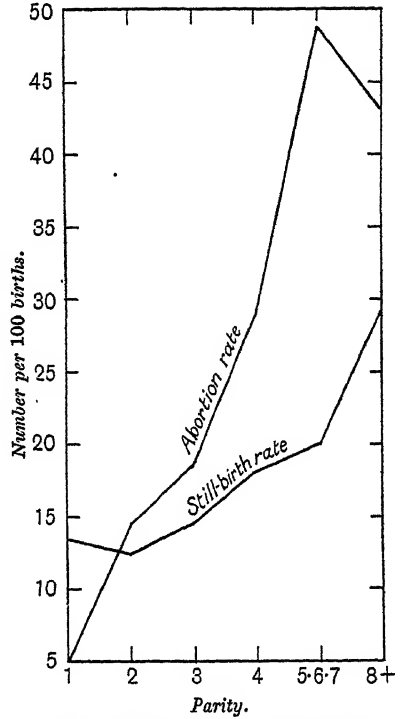


Fig. 2. Abortion and Still-birth rates at Parity groups.

### CONCLUSION.

It is thus very probable that the low sex-ratio found among later births and among the progeny of older mothers is accounted for by an increased amount of sexually differential pre-natal mortality. We may tentatively conclude, therefore, that the two influences here dealt with, exerted through the maternal organism of mammals, operate by unequal elimination of the sexes subsequent to sex determination, but previous to the time at which the sex-ratio is usually assessed. If this is the case, there is no inherent incompatibility between the mother having some influence on the birth sex-ratio and the male being the sex heterozygous, and therefore sex determining parent.

## SUMMARY.

1. There is an apparent discrepancy between the facts pointing to the male as the sex heterozygous parent in mammals, and the facts which show that the mother has some influence on the sex-ratio at birth.

Chief among the latter are those relating to the influence on the sex-ratio of the age of the mother and the number of the pregnancy.

2. The only sex-ratio, however, which we know to be affected by the mother is the ratio at birth, whereas the facts of sex determination are concerned solely with the ratio at the time of conception, or when sex is first determined.

3. It is possible, therefore, that changes in the sex-ratio between conception and birth may account for the apparent anomaly, and this would reconcile the two opposing sets of facts.

4. As changes in the sex-ratio after sex determination can only go on as a result of unequal elimination of the sexes the source of any change in the sex-ratio during gestation must be sought in a sexually differential pre-natal mortality.

5. Other authors have made it clear that there is a considerable amount of pre-natal mortality which falls more heavily on the males than on the females, and the material here presented shows that pre-natal mortality occurs preponderatingly with older mothers and in later pregnancies.

6. These facts substantiate the possibility that the decrease in the sex-ratio with older mothers and at higher parities is brought about as the result of increased pre-natal wastage of males, and not as the result of an increased proportion of females at sex determination.

7. Influences exerted by the mother, therefore, have no relation to sex determination, and offer no contradiction to the chromosome theory of sex determination, which implies that the male controls determination.

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# INHERITANCE IN BARLEY.

## III. THE AWN AND LATERAL FLORET (contd.): FLUCTUATION: A LINKAGE: MULTIPLE ALLELOMORPHS.

By F. L. ENGLEADOW,

*Plant Breeding Institute, Cambridge.*

(With eight Text-figures.)

### CONTENTS.

SECTION	PAGE
I. Introductory . . . . .	49
II. Awn Fluctuation . . . . .	51
III. Lateral Floret Fluctuation . . . . .	56
IV. Ear-width Fluctuation . . . . .	63
V. The Cross <i>H. decipiens</i> × <i>inermis</i> . . . . .	65
VI. An Awn-Lateral Floret Linkage . . . . .	69
VII. The Genetic Relationship of the Awn and the Lateral Floret in the Main Groups of <i>Hordeum sativum</i> . . . . .	73
VIII. Multiple Allelomorphism in <i>H. sativum</i> . . . . .	81
Appendix I. Some <i>H. distichum</i> × <i>H. distichum</i> Crosses . . . . .	84
Appendix II. A Case of Natural Cross Pollination in Barley . . . . .	85
Bibliography . . . . .	86

### I. INTRODUCTORY.

THESE observations are in continuation of others already published [Engledow (1) and (2)]. From the earlier work the main conclusions were:

(a) The several forms of lateral floret in *Hordeum sativum* appeared to have simple Mendelian modes of inheritance.

(b) In awned × awnless crosses awn-inheritance was in general of simple form but in one or two cases the possibility of complexity was revealed.

(c) Of these cases, one possessed special interest. The “six-row” habit and the “presence of awns” showed a linkage. Whether this was total or merely very high remained, after the inspection of considerable populations, still a matter of doubt.

(d) The four principal forms of lateral floret in *H. sativum* were regarded as constituting a multiple allelomorph series.

(e) Other investigations of awn and lateral floret inheritance had produced results out of harmony with the ones above described [(a)—(d)].

For example von Ubisch's theory involved three factors for the lateral floret.

(f) Fluctuation of the awn and the lateral floret was in some cases very pronounced. It seemed possible that in consequence multifactorial inheritance might be suspected where actually only one factor was concerned. Thus in one cross the  $F_2$  evidence warranted the postulation of three factors—one an inhibitor—whereas from the  $F_3$  unifactorial inheritance was established.

In continuing the investigation there have been four main objects. To learn more about fluctuation was the first. An example in ear-width was studied because of its intrinsic interest and economic importance. But attention was principally directed to the awn and the lateral floret. These two characters, in both the taxonomy and the genetics of *H. sativum*, have a high importance. Among the characters of that species they are the most emphatic and readily observable. Division into sub-species is based upon lateral floret form. It may therefore be said that further advance in barley genetics must await fuller study of the range and form of the fluctuation which these two characters display. The forms constituting *H. sativum* have distinctive genetic recommendations. All have the same low chromosome number (haploid = 7) and they may be freely intercrossed without sterility. On these grounds the awn and lateral floret have appeared to warrant a degree of attention which might at first sight seem undue. The causes of fluctuation present a separate problem. Invariably the late tillers of the plant are the most fluctuant. This suggested that as a first step the influence of "time of sowing" should be studied. Its precise physiological import lies beyond the scope of the investigation which aims simply at a determination of its relation to adult morphology. Certain barley forms and hybrids were distinguished by exceptional fluctuability. From these, selections of proved homozygosity, were taken for investigation.

For a number of reasons it was desirable to try to settle the nature of the linkage previously mentioned. This linkage was one of the evidences derived from awned  $\times$  awnless crosses which bore upon the view that the four chief lateral floret forms of *H. sativum* constituted a multiple allelomorph series. Full consideration of this view involved the fourth object of investigation which was an attempt to compare, and if possible co-ordinate, the different published results relating to awn and lateral floret inheritance.

A case of natural out-crossing in barley, believed to be a very rare phenomenon in England, is described in Appendix I.

## II. AWN FLUCTUATION.

In a previous publication [Engledow (2)] are recorded the facts concerning several crosses of awned and awnless forms. The awnless parent was an *inermis* form originally bred by Rimpau from the cross *H. decipiens* var. *steudelii*  $\times$  *H. hexastichum* var. *trifurcatum*. Invariably, in classifying the  $F_2$ , great difficulty was met. This could be lessened by restricting observation to median florets only and sorting on the basis of amount of awn borne by the outer paleae of these florets. Even this simpler grouping, however, was an uncertain process. Fully awned forms were readily distinguishable but the remaining plants of an  $F_2$  constituted a graduated series whose limits were marked by awns of about three-quarter length and complete awnlessness. Repeated examination of the series suggested that grading into three or four classes might be possible, e.g.  $\frac{3}{4}$  awn :  $\frac{1}{2}$  awn :  $\frac{1}{4}$  awn : no awn. This idea was supported by the published results of von Ubisch (3) and Ikeno (4) who effected a composite grading of awn-length and built upon it a multifactorial theory of awn inheritance. Put into practice for the  $F_2$ 's and  $F_3$ 's of these awned  $\times$  awnless crosses, however, such a grading proved impracticable, for repetition of the sorting failed to give consistent results. Moreover, in some cases, comparable crosses appeared to disagree. It seemed then that the grading was so arbitrary as to have no biological (or genetic) significance: in fact that the degree and kind of fluctuation induced by the environment afforded to an  $F_2$  or  $F_3$ , very largely determined the genetic ratios derived. Supplementary evidence to the same end was afforded by comparison of the individual ears of the  $F_2$  plants. Instances abounded in which most of the ears of a plant were completely awnless while a single, small, late-formed ear had quarter or even half awns. This fact brought under suspicion a number of poorly-grown plants mostly bearing but one ear, and which had been classified as half or quarter awned. Their immaturity at harvest showed that they had been late in development and it seemed possible that they were simply fluctuant forms of a normally awnless genotype. The *inermis* parent itself displayed a corresponding though less marked fluctuability in awn-length.

Since lateness of ear-development seemed associated with a tendency to produce "scurs" (short awns) it was decided to investigate the effects of late and early sowing. Later on it became especially desirable to try to demonstrate the degree of awn-fluctuability for by making careful allowance for its existence, consistent sorting of the  $F_2$ 's and  $F_3$ 's became possible. This sorting, moreover, pointed to straightforward unifactorial

awn-inheritance. Of a number of forms of *inermis* which had been carefully maintained for some years, that which seemed most fluctuable was selected for the variable time of sowing experiment.

From the 1920 harvest of this selected pure line, twelve good plants were taken. The total seed of every plant was divided at random into three parts. In this way were obtained three strictly comparable sets of seed each containing twelve lots. These sets were sown thus:

1st Set.....October 7th, 1920.

2nd Set.....March 15th, 1921.

3rd Set.....April 15th, 1921.

Soil, method of planting, and all other circumstances were as uniform as it was possible to make them on a well-kept trial ground. At harvest 1921, the plants of the first set were found to be much more robust and with plumper and more finely matured grains than those of the other sets. [It was a universal experience that in 1921, owing to the drought, all winter corn was better filled and matured than the spring.] Set three was slightly poorer than set two. In regard to awn-development the differences were very striking. The first set was completely awnless while sets two and three had, in general, awns of fully half length. Set three had slightly longer awns than set two but the difference was not noteworthy. The photographs of Fig. 1 illustrate the effects. On the left are four ears of set one, on the right four of set two. In both cases the four ears photographed were genuinely typical of the whole bulk. Not a single ear of set one displayed any trace of awn: in set two not a single ear was devoid of awns of about the length portrayed. Among the plants of set two, tiller to tiller differences were found. They were such as to emphasise the difference illustrated by Fig. 1. The method adopted—growing some of the seed of each of the 1920 plants in all three sowing sets—removes all doubts as to possible genotypic differences.

In 1921–2 the test was repeated. The best ten plants (i.e. those having most grain) of sets one and two of the 1921 harvest were used for seed. The complete bulk of seed from each of these twenty plants was divided at random into three parts. Thus were obtained from the October 1920-sown plants three sets of seed each of ten lots and from the March 1921-sown plants three precisely similar sets. The three sets of each of these two main groups were sown thus:

1st Set.....October 28th, 1921.

2nd Set.....January 30th, 1922.

3rd Set.....March 17th, 1922.

This plan again secured a safeguard against the influence of possible genotypic differences: and further it resulted in the sowing at three different times of grains from completely awnless and from half-awned plants of the 1921 harvest. At the 1922 harvest it was seen that the results were precisely those predictable on the view that time of sowing and awn-fluctuation are related in the manner suggested by the results of the previous year. Differences were not so well-marked as at the 1921 harvest, a fact which experience of several seasons is inclined to



Fig. 1.

attribute to the very pronounced rainfall and sunshine contrasts of the two years. Every plant in all three sets was carefully examined with the following results:

(a) Among the October 1921-sown plants from both sources [i.e. from the October 1920-sown and March 1921-sown progenitors] were three plants whose late tillers bore short awns. The extreme example was a plant from the October 1920-sown progenitor source. Its latest tiller bore awns of 1.0–4.0 cm. in length. For the rest, the whole of this group was completely awnless.

(b) The plants of both the January 1922-sown crops were either completely awnless or displayed, even on their earliest and best ears, awns of 1.0–2.0 cm. In some cases late tillers produced awns of 4.5 cm.

(c) All the plants of the March 1922 sowings were definitely “scurred” or half-awned. Not a single ear was awnless. As in (a) and (b), very late tillers were the most aberrant.

(d) Each of the populations described in (a), (b) and (c) consisted of the two groups grown respectively from the grains of October 1920-sown and March 1921-sown progenitors. At the 1922 harvest there was no evidence of an intergroup difference whether of awn-development or any other kind.

(e) Owing to the heavy rains of the summer of 1922, late tillers presented a special problem. In addition to the “late” tillers normally found, there was in these experimental populations, as in all field crops, a veritable “second crop.” On some plants two to five new tillers arose and flowered just before harvest. They set no grain but all bore awns of 1.0–5.0 cm.

From the evidence of the two seasons it appears that the critical sowing time in relation to awn-fluctuation lies probably in the month of February. Doubtless it is different in different seasons and habitats, but some interest may attach to its determination and an experiment to this end is in progress.

Another interesting instance of awn-fluctuation was afforded by an “awnless six-row” barley obtained from the East. Having grown badly in 1920 it was sown in two sets in 1921, the one in the open, the other in pots in a house. Both sets grew fairly well but both had awns of about quarter length. Figs. 2 (open-growing) and 3 (house-growing) show that the amount of awn was inconstant (e.g. Fig. 3 (*f*) is clearly awnless). Here then is a form, different from *inermis*, which while normally awnless in the East, obviously fluctuates when grown in England.

It is concluded that the so-called “awnless” barleys are very prone to fluctuation. The circumstances of the test described were naturally adjusted to give full scope to fluctuability but the results are such as to call for great caution in genetic work upon awn-inheritance. A simple classification which attempts to allow for considerable fluctuability seems far safer than a manifold one based upon several degrees of awn development. On the simpler basis, awns and awnlessness appear to represent a difference of one factor only [Engledow (2)].

It has seemed desirable to record the method and results at length because the facts of fluctuation can be established only by close attention

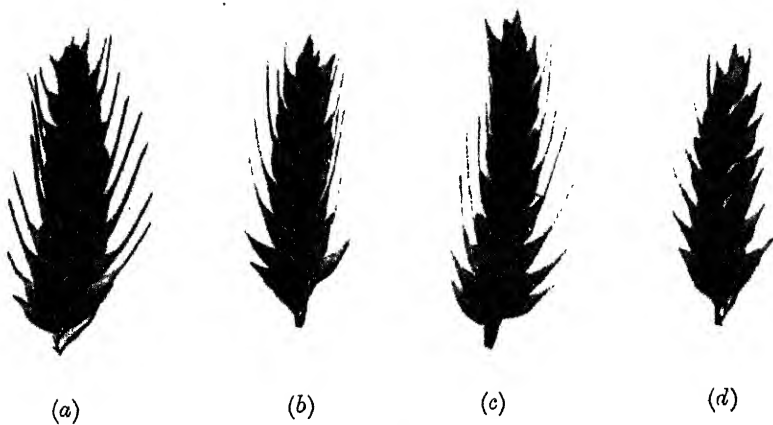


Fig. 2.



Fig. 3.



to detail. Among methods of meeting the difficulty of fluctuation in sorting  $F_2$ 's, etc. suggested in an earlier publication, was that of observing "co-fluctuants." If a character  $B$  could be found which fluctuated sympathetically with the experimental character  $A$ , suspected  $A$ -fluctuants might be tested by observation of  $B$ . Beyond the fact that lateness of development and a tendency to "scurs" were usually associated in awnless forms, no clue to a suitable co-fluctuant of the awn was found.

A curious interest attaches to the relation of awn-length and growth-period from the well-known fact that the awn is an important transpiratory organ. Its removal at ear-emergence [Harlan (5)] results in poorly filled grains. In awnless barleys however the amount of awn produced is greatest on the ears having the poorest grains. Presumably, whatever the relation of awn and grain-filling, both are profoundly affected by shortness of growth-period.

### III. LATERAL FLORET FLUCTUATION.

The taxonomic value placed upon the lateral florets of the cultivated forms of *Hordeum sativum* may be briefly expressed by an outline of classification :

*H. hexastichum* (6-row barleys) = Lateral florets all fertile (i.e. grain-bearing) but always smaller than the medians. Awn (or hood) development is precisely similar for laterals and medians.

*H. intermedium* (Intermedium barleys) = Lateral florets of variable fertility. Different proportions of fertiles: non-fertiles per ear exist as between different forms and upon this fact Harlan and Hayes (7) have succeeded in building a genetic classification adapted to certain specified circumstances. Whether fertile or not, the laterals relatively to those of *H. hexastichum* are markedly smaller than the medians, and they never bear awns or hoods.

*H. distichum* (2-row barleys) = Lateral florets never fertile. Very small differences in size appear to exist between the separate forms but they have not been considered to possess taxonomic significance.

*H. decipiens* (Abyssinian barleys) = Lateral florets extremely reduced. Two glumes and two diminutive paleae constitute the "floret."

Körnicker (6) in his classical barley observations recorded a number of instances of lateral floret fluctuation. These, like other "structural modifications" which he mentioned, appeared in some cases to be dependant upon time of sowing. Harlan and Hayes (7) in experiments which called for a very critical examination of the lateral florets of

hybrid progenies of *H. hexastichum*  $\times$  *H. distichum*, furnished striking examples of inter-tiller differences for a single plant (*loc. cit.* Plate CV, Fig. D). Similar examples, with a description of their genetic significance, have been given in a previous publication [Engledow (2), p. 178 and Figs. 1, 2, and 4]. The general experience has been that, as with the awn, fluctuation is most pronounced in plants of known hybrid origin and that its greatest intensity occurs on the latest-formed tillers. The solution of a number of interesting problems in barley-genetics has been



Fig. 4.

found to depend upon the correct interpretation of lateral floret fluctuation. This has pointed to the need for further study of the phenomenon in all the accepted "sub-species" of *H. sativum*. In every sub-species a considerable number of forms has been grown and in many cases the effect of different sowing-times has been tested. Instances of minor fluctuations have frequently been encountered but in the description which follows, mention is made of outstanding examples only.

Among a great number of forms of *H. hexastichum* there has been found only one which displays lateral floret fluctuation. It is to be noted

that in all forms an occasional infertile floret may be seen. But such florets have fully developed paleae, awns, and reproductive organs, and occur among the medians as frequently as among the laterals. Their infertility is accidental rather than developmental. The one exceptional form was sent from the highlands of Abyssinia by Mr G. W. Grabham of the Geological Survey of the Anglo-Egyptian Sudan. On almost every ear were one or two lateral florets of typical *decipiens* form. The rest of the florets were quite normal *hexastichum*. In three successive seasons this peculiarity has been maintained and it is therefore to be regarded as a constant and heritable attribute.

The forms of *H. intermedium* are outstanding in lateral floret fluctuation. As between different forms, floret-size is variable, but complete awnlessness of the lateral characterises all forms. The proportion of laterals which set grain is variable in such a manner as to have a restricted taxonomic value but in some forms it is decidedly fluctuable within the form. To test this matter, two sowings were made of a well-recognised pure line of *H. i.* var. *hawtoni*, the first on October 12th, 1920, the second on March 15th, 1921. At harvest 1921 the whole crop of each sowing was gathered and examined ear by ear. On every ear the number of median grains was counted and also the number of fertile (i.e. grain-bearing) lateral florets. Table I contains the result. In columns 1 and 2 are given the frequency-distributions (in percentages) of number of median grains per ear. Columns 3 and 4 show the average numbers of fertile lateral florets per ear for the classes of columns 1 and 2. The numbers of ears counted were, for the October 1920 sowing 181 and for the March 1921 sowing 82. Between the crops two differences are revealed in the table. From a comparison of columns 1 and 2 it appears that the modal (and mean) number of median grains per ear is greater for the October sowing. The actual mean values are October = 22.8 median grains per ear and March = 21.1. Similarly it is seen from columns 3 and 4 that the average number of fertile lateral florets per ear is greater for the March sowing in almost every frequency class of the table. In most classes it is markedly greater and for the complete populations the mean numbers of fertile lateral florets per ear are October sown = 1.4 per ear, March sown = 2.7 per ear. It is evident then that in this particular case an earlier sowing, which involves a longer growing period, leads to the formation of a greater average number of median (and therefore lateral) florets per ear. But of the laterals formed, the actual number, and still more the proportion, which achieve fertility, is less for the early than the late sowing. The general nature

TABLE I.

*Frequency distribution of single ears according to number of median grains per ear: and also the average number of fertile lateral florets per ear for every class of the distribution.*

The frequencies are expressed as percentages of the total number of ears.

	1	2	3	4
Number of median grains per ear	Percentage of total ears for Oct. 1920, sown	Percentage of total ears for March 1921, sown	Average number of fertile laterals per ear for Oct. 1920, sown	Average number of fertile laterals per ear for March 1921, sown
9	1.1	2.4	—	0.5
10	0.6	2.4	—	—
11	2.8	1.2	—	—
12	1.6	—	—	—
13	0.6	1.2	—	—
14	2.2	2.4	1.8	—
15	2.2	4.9	0.5	—
16	1.7	1.2	0.3	1.0
17	3.3	1.2	0.5	—
18	3.9	2.4	—	4.0
19	6.0	7.3	0.6	1.7
20	5.5	4.9	—	2.0
21	4.4	18.3	0.1	2.8
22	7.7	17.1	1.3	3.2
23	3.9	9.8	0.3	3.3
24	7.1	7.3	0.9	3.5
25	8.3	3.7	0.9	4.7
26	8.8	3.7	2.8	2.0
27	6.6	2.4	3.6	6.5
28	8.8	3.7	2.3	5.0
29	3.3	1.2	5.3	11.0
30	4.9	1.2	2.8	4.0
31	2.8	—	3.0	—
32	1.7	—	2.3	—

of this effect harmonises with observations repeatedly made upon both *H. i. hauxtoni* and *H. i. transiens*.

It is noteworthy that in both sowings, separately considered, as the number of median grains per ear increases, the number of fertile laterals increases. Since there are twice as many laterals as medians this result is to be expected on the ground that the more laterals formed the greater the number likely to be fertile. As between the averages for the separate sowings, however, a different relationship holds. The early sowing produced a greater number of lateral florets per ear but a smaller number of fertile ones. It follows that lateral floret fertility is influenced by time of sowing in a different manner from size of ear (as measured by number of florets formed). From this it is inferred that the fertility of the laterals of *H. i. hauxtoni* is in large measure an expression of mechanical rather than developmental influences. Probably all the lateral florets are potentially fertile but for positive information microscopic study is

required. At first sight the facts appear out of harmony with the view that average percentage lateral fertility can in some circumstances be employed in genetic classification. This is not the case, however. Among the forms of *H. intermedium* there are undoubtedly broad groups whose percentage lateral fertilities, though very fluctuant, are characteristically different. It is clear, however, that the genetic position of a form of *H. intermedium* cannot safely be based upon percentage lateral floret fertility unless a full statistical test of the percentage is made.

In *H. distichum* minor differences in the lateral floret may be observed but they have never been employed taxonomically.

The safest general statement is probably that the broad ear (dense rachis) forms as a whole have larger, more inflated laterals than the narrow ear (lax rachis). Small as these differences are they yet have a genetic interest. A series of crosses of *H. distichum*  $\times$  *inermis* (an awnless form whose laterals are usually semi-decapiens but may occasionally fluctuate to almost *distichum* size) has already been described [Engledow (2), pp. 185-6]. It was found that "in Cross E. 32 [Chevallier (narrow ear)  $\times$  *inermis*] little range of lateral floret size was shown in fully-awned extracts, but in other crosses (the awned parents of which were broad-eared) distinct differences appeared. Density of ear clearly segregated, and the denser the ear the larger the lateral florets." The progenies of these crosses have now been observed to the  $F_3$ 's. There is no doubt about the existence of minor lateral floret differences of the kind to which the earlier publication refers, but fluctuation appears to be too general and too pronounced to permit of certain discrimination. All the  $F_2$ — $F_3$  types studied, displayed the two familiar phenomena—inter-floret differences on the ear and inter-tiller differences on the plant. In contrast to these extracted types, the cultivated forms of *H. distichum* appear to be singularly constant in the lateral floret. The least constant is, perhaps, the form known as "Plumage" which is much grown in England. In the summer of 1921 ears were found on which were some very much inflated laterals: but in no case in this, or in any other recognised *distichum* form, was a fertile lateral floret seen.

The forms of *H. decipiens* as a whole are marked by singular constancy of lateral floret form. A striking exception was found, however, in two specimens sent from Abyssinia by Mr Grabham. They displayed a fluctuation complementary to that of the *hexastichum* from the same source described above. The early-formed ears of the plant were usually normal *decipiens* [e.g. Fig. 7 (a)] but in a few cases such ears had one or two *hexastichum* laterals [e.g. Fig. 7 (b)]. Almost invariably the late tillers

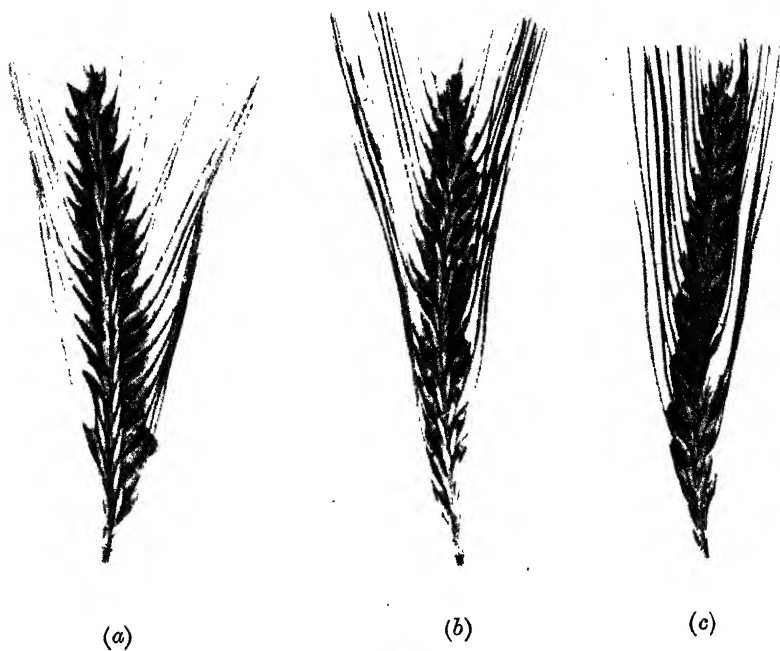


Fig. 5.



Fig. 6.

had laterals all of *hexastichum* form and developed grains. The actual inter-tiller differences are illustrated by Figs. 6 and 7, each of which represents the ears of a single plant. In all cases the aberrant *hexastichum* laterals were as fully awned as the medians (all awns were removed to simplify the photographs). It is necessary to point out that the phenomenon described is in no way related to the duplication of the median grains, a familiar and non-heritable teratology. Mr H. V. Harlan, Agonomist in Charge of Barley Investigations, Bureau of Plant Industry,

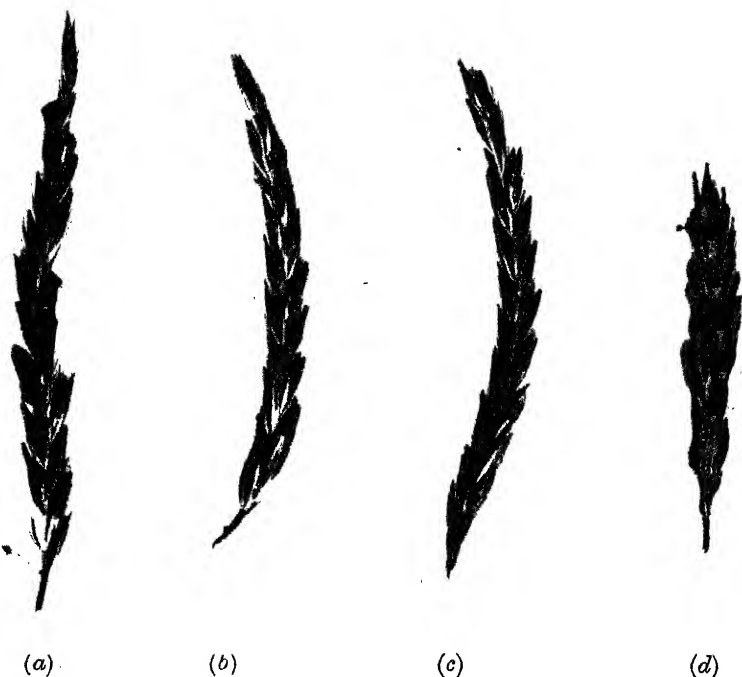


Fig. 7.

Washington, has very kindly furnished some information concerning what are evidently similar forms from Abyssinia. Grown in America, they have constantly displayed fluctuant *hexastichum* laterals and soil condition has been proved to influence the extent of the fluctuation.

It is clear that lateral floret form in *H. sativum* is complex. Obscure factors producing small visible effects probably exist, and some of them are almost certainly linked with other characters such as those controlling rachis density. Fluctuation is of general occurrence and at present on the one hand prevents full factorial elucidation while on the other it may

suggest multi-factorial inheritance where this does not exist. One form of fluctuation described has an interesting bearing upon the multiple allelomorphism which has been attributed to the series of lateral floret forms. If these forms be simple gradations, i.e. a purely quantitative series, their multiple allelomorphism is not a striking phenomenon. But there seems to be *prima facie* justification for expecting fluctuation in such a graded series, itself to display gradation. For example, if the *H. decipiens* form of floret fluctuates in the direction of the other forms, it might be expected, in a proportion of cases, to reach the *H. distichum* or *H. intermedium* form. In the fluctuant *H. decipiens* from Abyssinia, however, the inconstant lateral florets invariably reached *H. hexastichum* form. Correspondingly the fluctuant *H. hexastichum* in all cases reached *H. decipiens* form in its inconstant laterals. These facts do not accord well with the quantitative series acceptance. Fluctuation from *H. decipiens* to *H. distichum* form has, however, been observed in a few segregates from *H. distichum*  $\times$  *inermis* crosses. From the point of view of the quantitative nature of multiple allelomorphism, further study of lateral floret fluctuation might well be repaid.

#### IV. EAR-WIDTH FLUCTUATION.

Agriculturists classify the forms of *H. hexastichum* into "six-row" and "four-row" types. These correspond to the dense rachis and lax rachis types of the botanist. Correspondingly the forms of *H. distichum* are distinguished as "broad-ear" and "narrow-ear." To simplify discussion, only the *distichum* forms will be considered: the principles enunciated apply, however, to the *hexastichum* barleys as well. A broad-ear barley is one with a dense rachis i.e. with short rachis internodes. In general the shorter the internodes, the greater the crowding of the grains, and consequently the higher their inclination to the rachis and the greater the "width" of the ear. "Width" is, of course, the average distance between the two lines defined by the tips of the grains on the two sides of the ear. Ear-width is a character commonly employed in practical plant breeding. It is always estimated by eye and with experience, small differences in its magnitude are readily distinguished. The associated character—rachis density—is usually measured. Average internode length for the rachis is the simplest form of measurement but in genetic work the more refined method of Hayes and Harlan (8) is preferable. These investigators have shown that rachis density, as they measure it, is fairly constant in the barley



forms with which they worked. It has been found [Percival (9) p. 160] that the different forms of wheat are variable in the constancy of their rachis density.

During the period 1919-22 a great number of barleys, segregates from crosses between established malting forms, have been studied for economic purposes. The principal characters observed were yielding capacity, tillering, straw length and strength, shortness of "neck," and malting quality. Close attention was also devoted to botanical characters to ensure that the final selections should be as fully homozygous as possible. Of these botanical characters by far the most elusive has been ear-width. The great number of the breeding forms and the diversity of agricultural and botanical characters which had to be observed, made it necessary to trust to eye-judgment of ear-width. Some forms, very satisfactory in all other respects, appeared to contain different ear-width types. Further selection failed to establish fixity, slight but definite differences appearing year after year. It came to be realised that much if not the whole of the difficulty lay in the fact that the "outside" plants (plants of end rows and end plants of other rows) always had narrower (laxer) ears than inside plants. In other words decrease in ear density was one of the features of the increased vigour of growth which always characterised "outside" plants.

As the question was one of considerable practical importance it was closely studied. Particular attention was paid to inter-tiller differences on the same plant. Such differences were expected and were found. Their magnitude differed in the various commercial barley forms observed. In one it was very striking and it will suffice to describe the facts relating to this one form. Any crop of it, particularly when the grain is fully swollen but still green, appears to contain ears of very different widths. So much is this the case that doubts have often been expressed as to its homozygosity in ear characters. The range of width is, indeed, in the green state, from that of a broad-ear to that of a narrow-ear barley. It was observed, however, that among the tillers of a single plant there might be ear-width differences of the same order. Moreover, daily observation of single ears showed that the ear-width steadily increased as the grains developed. This must necessarily happen, of course, in every barley, but the increase was particularly pronounced here. In this fact appeared to lie the explanation of the comparative inconstancy of ear-width which the form displayed and which was in contrast with the results of Hayes and Harlan on rachis density already mentioned. Compared with most two-row barleys, this

form proved to have a rather exceptional rachis. The attachments of the median grains were smaller and more inclined than is general. Consequently as the grains increased in weight, they became more and more obliquely inclined to the rachis, the ear-width being thus steadily increased. Harlan (10) has shown that the wet weight of the developing barley grain increases very rapidly. On account of the rachis peculiarity described, this increase markedly affects the ear-width. Fig. 5 illustrates three ears (*a*), (*b*) and (*c*) from one plant at maturity. In comparison with other barleys (*a*) would be classed as broad-ear and (*c*) as narrow. It will be noticed [e.g. ear (*a*)] that adjoining grains do not touch. The forcing outwards of the grains in this case thus reflects simply the influence of gravity and not, as in true broad-ears, inter-grain pressure for which density of rachis is responsible.

This form is certainly exceptional in not closely reflecting rachis density in its ear-width, as also in its fluctuability. An account of it has appeared desirable because of the plant breeding importance of ear-width. From an imperfect understanding of this character much waste of time and loss of useful material may result. To the same cause are to be attributed, possibly, some of the "new and slightly different" barley forms whose distinction from old ones is not in all circumstances certain. In oats the shape of the panicle presents a similar problem of statics. Most of our commercial forms are hybrids of *A. sativa* and *A. orientalis* of "intermediate" panicle shape. This shape changes with development of the grain in the way explained. Distinctions based upon small shape differences are consequently in most instances of no value.

#### V. THE CROSS *H. DECIPIENS* × *INERME*.

This cross is an interesting extension of the group of *inerme* crosses described in the second paper of this series [Engledow(2)]. The outstanding features of the parents are, briefly, these. *H. decipiens* has fully awned median florets but its laterals are extremely reduced and devoid of all trace of reproductive organs. As Fig. 8 (*a*) shows, there are two glumes of normal size and two extremely small paleae. The *inerme* parent has awnless median florets and its laterals may be described as semi-deciapiens. They are completely devoid of reproductive organs and definitely smaller than the *distichum* lateral type: but they are always of greater length than the true *decipiens* [vide Fig. 8 (*c*)]. In addition *inerme* has a much denser ear than the *H.*

*decipiens* parent and very occasionally one or more lateral florets upon an ear may fluctuate to *distichum* dimensions or even attain fertility. The occurrence and significance of such fluctuations of *inermis* and its hybrid offspring are fully considered in the paper above mentioned. No significant fluctuation has ever been observed in the lateral florets of the *H. decipiens* form used as a parent in this cross.

The  $F_1$  plants bore awns of about half length. Upon the single ear awn length was very fluctuante and the late tillers on all the plants had longer awns than the early. Lateral floret form was a parental blend and displayed no noteworthy fluctuation. In  $F_2$  there were 1247

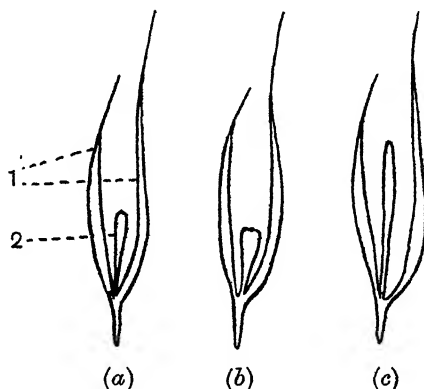


Fig. 8. Lateral floret forms in the cross *H. decipiens*  $\times$  *inermis*.

(a) = *H. decipiens*  $F_0$ . (c) = *Inermis*  $F_0$ . (b) = An exceptional  $F_2$  type.

1 = glumes. 2 = paleae.

plants in ten families [A—K in Tables II and III] each of which was the progeny of a single  $F_1$  plant. Classification upon the basis of amount of awn was first essayed and it proved to be singularly simple. All the plants were either fully awned like the  $F_0$  *H. decipiens* or completely awnless. There is one reservation to this general statement but it has no genetic significance. Of the 965 awnless plants 11 had one or more small late tillers upon whose ears were "scurs" from 0.5–1.5 cm. in length. In every respect they resembled, and may safely be regarded as equivalent to, the occasional fluctuant "scurs" of the late tillers of the *inermis*  $F_0$ . Assuming the awnless population to comprise both homozygous and heterozygous forms it may, in conformity with the notation used for the other *inermis* crosses, be denoted by  $(\beta + \gamma)$ . Correspondingly  $(\alpha)$  may denote the fully awned population. Table II shows the proportion  $(\alpha) : (\beta + \gamma)$ . For the complete  $F_2$

TABLE II.

The  $F_2$  of *H. decipiens*  $\times$  *inermis* classified on awns into ( $\alpha$ ) = fully awned.  
 ( $\beta + \gamma$ ) = completely awnless. A—K = separate  $F_2$  families.

$F_2$ family	Number of Plants	Percentage		Percentage Deviation from 1:3 Expectation	Percentage Errors of Sampling
		( $\alpha$ )	( $\beta + \gamma$ )		
A	178	21.9	78.1	3.1	3.25
B	89	21.3	78.7	3.7	4.59
C	73	17.8	82.2	7.2	5.07
D	100	19.0	81.0	6.0	4.33
E	140	27.8	72.2	2.8	3.66
F	134	28.4	71.6	3.4	3.74
G	162	25.3	74.7	0.3	3.40
H	136	20.6	79.4	4.4	3.71
J	97	23.7	76.3	1.3	4.40
K	138	16.7	83.3	8.3	3.68
Total	1247	22.61	77.39	2.39	1.23

TABLE III.

The  $F_2$  of *H. decipiens*  $\times$  *inermis* ( $\beta + \gamma$ ) Group (of Table II) classified on lateral florets form into I. non-decipiens and II. decipiens. A—K = separate  $F_2$  families.

$F_2$ family	Number of Plants	Percentages		Percentage Deviations from 1:3 Expectation	Percentage Errors of Sampling
		I	II		
A	139	23.02	76.98	1.9	3.67
B	70	20.00	80.00	5.0	5.17
C	60	23.33	76.67	1.7	5.84
D	81	25.92	74.08	0.9	4.81
E	101	24.75	75.25	0.3	4.31
F	96	26.04	73.96	1.0	4.42
G	121	26.44	73.56	1.4	3.94
H	108	23.14	76.86	1.9	4.17
J	74	22.97	77.03	2.0	5.03
K	115	27.82	72.18	2.8	4.04
Total	965	24.56	75.44	0.14	1.39

the value is 22.61% : 77.39% which represents a deviation of 2.39% from the 1:3 expectation. Since the standard error attributable to errors of sampling is only 1.23%, this deviation (= 1.95 S.E.) though statistically acceptable, must be regarded as high. In the separate  $F_2$  families, marked ratio differences occur. From the data of Table II it may be seen that in four families the deviation is less than the S.E. (standard error due to sampling), in five it lies between 1.0–2.0 S.E. and in one it is 2.25 S.E. This is rather unsatisfactory as is shown by

comparison with the expectation calculated from Pearson's Tables of Probability Integrals.

Deviation in terms of s.e.	Number of Deviations Observed	Number of Deviations Expected
0.0—1.0 s.e.	4	6.8
1.0—2.0 s.e.	5	2.7
> 2.0 s.e.	1	0.5

A further test of goodness of fit in the separate families may be applied by calculating the s.e. of the percentage of fully-awned plants for a sample of size  $H$  [ $H$  = harmonic mean of frequencies of families]. This error is 4.02 %, whereas the s.e. of the actual percentages in the families is 3.81 %. By this, as by the other tests, the 1 : 3 ratio is thus vindicated but not with the emphasis desirable in work of this kind.

The next step was to classify the awn-groups ( $\alpha$ ) and ( $\beta + \gamma$ ) on lateral floret form. Group ( $\alpha$ ) offered no difficulty for none of the plants showed a significant difference from the  $F_0$  *H. decipiens*. The identity was indeed, striking, and covered not only awn and lateral floret but apparently, ear density as well [*vide supra*, *H. decipiens* has an obviously laxer ear than *inermis*]. In crosses among different lateral floret forms there appear to occur inevitably small fluctuations whose characteristic form invites attention but to which no genetic significance can be accorded. The ( $\alpha$ ) group was no exception to this, for upon some ears there were two or three laterals, always situated near the middle of the ear, of the shorter and more inflated form illustrated by Fig. 8 (*b*). Careful examination of their nature and occurrence suggested that despite their distinctive difference from the  $F_0$  *H. decipiens* form [Fig. 8 (*a*)] they were to be regarded simply as fluctuants.

Group ( $\beta + \gamma$ ) was strikingly different from group ( $\alpha$ ) for it clearly displayed segregation of lateral floret form. The classification which finally proved practicable was :

- (I) Types other than  $F_0$  *H. decipiens*.
- (II)  $F_0$  *H. decipiens* type.

In I was included, apparently, a variety of types. On some plants the ears had laterals uniformly of *inermis* type [Fig. 8 (*c*)]. Other plants showed inter-ear differences and also inter-floret differences for the same ear. A few ears bore 1–3 fertile lateral florets the grains of which were very small. These florets resembled in all respects the occasional fertile laterals found in  $F_0$  *inermis*. Table III gives the values of the I:II ratio in the separate  $F_2$  families with the appropriate deviations and errors of sampling. For the complete  $F_2$  the ratio is

24.56% : 75.44%, a very close approach to 1 : 3 and in no family is the deviation greater than the S.E. of sampling. This latter fact is singular for theoretically a certain number of deviations in excess of the S.E. is to be expected.

Upon the results of the two-fold classification—by awn and by lateral floret—a number of alternative theories of inheritance might tentatively be built. No finality is possible, of course, without the raising of a complete  $F_3$ . The  $F_2$  result has been given, however, because it vouches for two facts :

(i) In contrast with other *inerme* crosses e.g. *H. distichum*  $\times$  *inerme* there are no half-awned forms in  $F_2$ .

(ii) Fully awned  $F_2$  plants [Group ( $\alpha$ )] show no segregation of lateral floret form : but such segregation is quite clear in the awnless forms [Group ( $\overline{\beta + \gamma}$ )].

These facts play an important part in the considerations to which §§ VII and VIII (*infra*) are devoted.

## VI. AN AWN-LATERAL FLORET LINKAGE.

Few linkages have been discovered in the cereals. All the recorded cases for barley are comparatively recent and based upon such data as makes the calculation of cross-over percentage impossible. v. Ubisch appears to be the first to have demonstrated the phenomenon in barley. The characters were [v. Ubisch(11) and (12)] “Zähnen” (small spicular projections) on the “nerves” of the outer palea and “six-row” (in contrast to “two-row”) habit. He found a second linkage between “long” beard and “lax” ear [v. Ubisch(12)]. Vavilov(13) discovered that in some cases “smooth awns” and “adherent paleae” were linked, while in others they were not. He pointed out, too, the possibility of linkage relationships connected with ear shape and confirmed, in principle, the first-mentioned instance of v. Ubisch. That certain “chlorophyll-factors” exhibit linkage has been reported by Nilsson-Ehle(14) although the progress of his investigation permits only a tentative statement. A paper in Japanese by Kiichi Miyake and Yoshitaka Imai(15) contains evidence [*vide* English Abstract] of the existence of two linkage groups. The comparative infrequency of linkages and their uncertain nature, in barley, appears to warrant the description of an apparant additional example which, like previous ones, is vague.

The possibility of an awn-lateral floret linkage in the cross *H. hexastichum*  $\times$  *inerme* has already been indicated [Engledow(2) pp. 181–2].

For a clear presentation of the additional evidence it is necessary to recapitulate the facts previously stated. Two forms of *H. hexastichum* were used in the crosses with *inerme*. The one was *H. h.* var. *parallelum* which has a dense ear: the other *H. h.* var. *praecox* of which the ear is lax. *inerme* has been described in § V (*supra*). From the  $F_2$  of *parallelum*  $\times$  *inerme* the only reliable information derived was six-row fully awned: other types = 23.4%:76.6%. This appeared to imply that one factor governed "full awn" plus "six-row habit." The slight defect of six-row fully awned plants while not militating statistically against the acceptance of the 1:3 ratio, might conceivably indicate two highly linked factors instead of a single one. A considerable  $F_3$  was raised but there was still no further suggestion of such a linkage.

Very similar  $F_2$  results were obtained from *praecox*  $\times$  *inerme*. The ratio six-row fully awned: other types was 23.84%:76.16%—again a slight defect of the first type of plant. More detailed classification proved impossible owing to the fluctuation of florets upon the ear and of the ears upon the plant. A complete  $F_3$  of 505 families was raised and the families sorted into the following categories:

( $\alpha$ ) All plants like *praecox* with full-size lateral grains and full-length awns.

( $\beta$ ) Plants of three types, viz. ( $\alpha$ ), ( $\gamma$ ), and a variable type, of intermediate awn length and with variable numbers of fertile lateral florets. This form displayed great floret-to-floret fluctuation on the single ear.

( $\gamma$ ) All plants as awnless as *inerme*. Most of them had, too, typical *inerme* laterals. On some, however, all the laterals were inflated but non-fertile, while a few bore fertile laterals. The number of these fertiles varied from one to almost the whole number.

The ratio was ( $\alpha$ ):( $\beta$ ):( $\gamma$ ) = 23.76:51.29:24.96(%) and it accords well with the corresponding ratio obtained from the  $F_2$  plants, viz. ( $\alpha$ ):( $\beta + \gamma$ ) = 23.84:76.16(%) displaying to practically the same extent a defect of ( $\alpha$ ) type. Statistically it is in close agreement with 1:2:1 so that the unifactorial inheritance of awn-cum-fertile-lateral seemed certain.

Three circumstances, however, appeared to be opposed to this:

(1) The small defect of ( $\alpha$ ) plants (six-row and fully awned).

(2) In one  $F_3$  family were found three "fully awned two-row" plants. Their lateral florets were of typical *H. distichum* form. No such plants were found in  $F_2$  and the proportion in  $F_3$  was only 3/4000. It seemed possible that the presence of these plants was due to the accidental admixture of three foreign grains but, against this, they appeared not

to be identical with any of the *H. distichum* forms which were being grown. They might be considered as evidence of a very high linkage between "six-rows" and "full-awns." But the awned cross-over type naturally expected should have *inerme* laterals and not those characteristic of *H. distichum*. From this difficulty, in turn, an escape might perhaps be offered by the familiar fluctuability of the *inerme* form lateral and the fact that lateral floret development appears in some circumstances to be governed by other characters which are present [e.g. the differences between  $F_2$  of Broad-ear *distichum*  $\times$  *inerme* and Narrow-ear *distichum*  $\times$  *inerme* in § III].

(3) In  $F_3$  ( $\gamma$ ) families, as described above, were a few plants on which most of the laterals were fertile. The grains were very small compared with the medians. In fact the ears resembled "awnless intermedium" rather than "awnless six-row." If, however, the suggested developmental influence of awn upon lateral floret were accepted, then these forms might represent the "awnless cross-over" type of a linkage.

From (1), (2) and (3) a case for a high full-awn : six-row linkage might be made out. Growings made in the following year to test this possibility gave results which are described seriatim below.

(A) The three fully-awned two-row plants bred true.

(B) A selection of the plants from  $F_3$  ( $\gamma$ ) families of high lateral floret fertility gave variable results. All gave entirely awnless progeny but whereas some of these had from 0-10 fertile laterals per ear, all laterals being of greater size than *inerme*, others were practically of *inerme* type.

(C) All the plants of  $F_3$  ( $\beta$ ) families were grown on. There resulted over 30,000  $F_4$  plants and among them was but one fully awned form of other than the  $F_0$  full-awn six-row type. This was of *H. distichum* type and closely resembled the three doubtful plants of the previous year save for its slightly greater ear density.

Finally, a year later, small  $F_5$  growings were made. They proved the homozygosity of the awned two-row types (3 plants in  $F_3$  and 1 in  $F_4$ ) and also of some of the "awnless six-row" (which as stated really resembled "awnless intermedium") referred to in (3) and (B) above.

The combined evidence afforded by  $F_2$ — $F_5$  is considerable but far from decisive. On the whole it is concluded that the characters "six-row" and "full-awns" are governed by separate but highly-linked factors. This conclusion, it must be re-affirmed, rests upon an assumption. Awn development must be supposed to influence lateral floret form and fertility. There is supporting evidence for this in independant crosses.



In connection with the acceptance of the linkage this influence must be held responsible for the smallness of the lateral grains on plants regarded as "six-row awnless" and the super-*inermis* size of the laterals on the awned cross-over type (i.e. the plants resembling, as described, ordinary *H. distichum*).

The theory of inheritance would therefore take the following form:

$A$  = factor for awns.

$S$  = " " six-rows (fertile).

$A$  and  $S$  are so related that awns and fertile laterals each attain perfect development only in the combination  $AA.SS$ .  $A$  and  $S$  are very highly linked so that in the cross  $AA.SS \times aa.ss$  the gametes of  $F_1$  ( $= AaSs$ ) are produced in the proportions  $(n-1)AS : As : aS : (n-1)as$ . The sorting of  $F_2$  would give the result:

$F_2$  = fully awned six-row : Intermediate forms : awnless forms

$(n-1)^2 AA.SS$  :  $[2(n-1)^2 + 2] AaSs : (n-1)^2 aa.ss$ .

Types  $aaSS$  (awnless six-row) and  $AAss$  (awned two-row) will be extremely rare.

Types  $2(n-1)AASs$ ,  $2(n-1)AaSS$ ,  $2(n-1)Aass$ , and  $2(n-1)aaSs$  would probably be placed in part with the Intermediate forms and in part (e.g.  $aaSs$ ) with the awnless forms.

Clearly the proportion of fully-awned six-row plants in  $F_2$  will, if  $n$  be high, fall slightly below 25% as was found to be the case and the  $F_2$  groups will approximate in proportion to 1 : 2 : 1. On this theory the  $F_4$  derived from the ( $\beta$ ) families [described in ( $B$ ) above] should contain a higher proportion of  $AA.ss$  (awnless two-row) than was found (viz. 1/30,000).

It is sometimes stated that "awnless six-row" forms of barley occur in Japan. Until quite recently opportunity had arisen to obtain specimens of only one of these. This form was described in § II (*supra*) in connection with awn fluctuation. It is illustrated in Figs. 2 and 3. The occasional occurrence of short awns is not inconsistent with the descriptive term "awnless" as here used but the distribution of such fluctuant awns is important. They never occur (*vide* Figs. 2 and 3) on lateral florets. From this fact it seems inevitable to conclude that this form is not a "six-row" barley but simply an "awnless intermedium." [*H. intermedium*, in its familiar awned forms, has fully awned medians and completely awnless laterals.] This view of the nature of the reputed "awnless six-row" barley of Japan destroys, of course, what at first seemed to be a piece of evidence for the belief that full awn and six-row

habit are merely partially linked. Since observations on this one form began, Mr H. V. Harlan has very kindly sent a number of such reputed "awnless six-row forms" from Japan and China. He has classified them all as *H. intermedium hastoni* var. *tonsum* [vide Harlan(21)].

#### VII. THE GENETIC RELATIONSHIP OF THE AWN AND THE LATERAL FLORET IN THE MAIN GROUPS OF *HORDEUM SATIVUM*.

The considerable body of evidence bearing upon this relationship is interesting for several reasons. It represents the work of some half-dozen investigators whose conclusions are not fully harmonious even on fundamental points. In the taxonomy of *H. sativum* the two characters—awn and lateral floret—are pre-eminently important. But perhaps the chief interest lies in the fact that the lateral floret forms which define the main groups of the species, are usually considered to constitute a simple "quantitative series" or progression. As such, they may be expected to behave on crossing in pairs, in the manner which has evoked the term "multiple allelomorphism." That they do so behave has been stated [Engledow(1) p. 106]. There is evidence, however, of complications which give an unusual significance to "multiple allelomorphism." The form of the complications is an apparently variable relationship between awn and lateral floret in the different groups of *H. sativum*. Characters constituting a simple quantitative series might all be expected to show constant or strictly analogous relationships to other characters. This expectation and the general form of the relationship may be explored by a brief review of genetic results for the inter-group crosses in turn.

##### (1) *H. hexastichum* × *H. distichum*.

This inter-species or inter-group cross has been more fully investigated than any other. The results of the separate experiments may be thus briefly stated.

(a) v. Ubisch(12) found the  $F_1$  to have laterals with short awns and to be occasionally fertile. In  $F_2$  the ratio 6-row : remainder was 1 : 3. This suggested a single factor for all the attributes of the lateral floret—awn, size, and fertility. But the plants other than normal 6-row constituted a gradation in awn, size, and fertility of the lateral floret which practically covered the inter-parental range. For this reason it was decided to attempt a 2-factor theory. Factor *Z* was proposed for the 2-row habit and an intensifier *W*, operative only in presence of *Z*, which is required to produce the full reduction of awn, size, and fertility

characterising the 2-row lateral floret. The data was not in very close agreement with expectation for the smaller groups [*vide* Engledow(1) pp. 102-3]. It is noteworthy that in both the unifactorial and the bifactorial explanations, the lateral floret is accepted as a unit—awn and other attributes are inseparable.

(b) Blaringhem(16) obtained results clearly in general accord with other investigators but he has placed upon them an interpretation of "mosaic" inheritance which cannot well be compared with the other evidence pertaining to this cross [*vide* Engledow(1) pp. 106-8].

(c) Biffen(19) has recorded a 1 : 3 ratio for 6-row : remainder in this form of cross and in a later publication [Biffen(18)] has given the data of a second similar result. In his view therefore, one factor controlled the size, fertility, and awn, of the lateral floret.

(d) Engledow(1) and (2). Evidence was obtained from seven different crosses and in every case the  $F_2$  ratio appeared to be 1 : 2 : 1. All  $F_2$  plants belonging to neither of the parental types were therefore regarded as being heterozygous for the single factor concerned. In every cross, such plants covered a considerable range of lateral floret development. It seemed satisfactory to attribute this fact to fluctuation and the  $F_2$  evidence accorded with the idea of a single factor. The proportion of 6-row plants in  $F_2$  ranged from 21.6% to 26.7%. It appeared not to be characterised by a small deficiency [cf. *H. hexastichum*  $\times$  *inermis* in (6) (a) below] so that there was no suggestion of a linkage.

(e) Harlan and Hayes(7) obtained from a cross of this type a barley form belonging to the group *H. intermedium*. They point out that other investigators have not done this and fully review the interesting question which their work raises. Two other recent investigators [Kezer and Boyack(17)] specifically report that they have been unable to obtain *H. intermedium* from the cross *H. hexastichum*  $\times$  *H. distichum*. The theory propounded by Harlan and Hayes is very important. They postulate an "epistatic" factor *A* and a "hypostatic" *B*. For the forms concerned in their work the following genetic constitutions were indicated:

$$\begin{array}{ll} H. hexastichum & \left\{ \begin{array}{l} \text{first form} = AA.BB \\ \text{second form} = AA.bb \end{array} \right. \\ H. intermedium & = aa.BB \\ H. distichum & = aa.bb. \end{array}$$

There was evidence of a third factor affecting lateral floret fertility. This theory fully accords with the experimental data. That two forms

(*AA.BB* and *AA.bb* in the above scheme) of *H. hexastichum* occur, is borne out not only by the numerical data of this investigation, but by a fact which the authors cite (*loc. cit.* p. 577) "other crosses were studied in which no other intermediates were produced." It seems possible that in the occurrence of two separate forms of *H. hexastichum* lies the explanation of the unifactorial ratios mentioned in (b), (c), and (d) above. And further, the bifactorial scheme of (a) above might conceivably be moulded into conformity with that suggested by these authors.

Fertility of the lateral floret was the prime experimental character: but size and awn development serve in the classification. Briefly, it may be said that factor *A* controls the awn, the size, and the fertility of the lateral floret of *H. hexastichum* while *B* controls the size and fertility (there being no awn) in the lateral floret of *H. intermedium*.

From the various results considered it seems safe to conclude that (i) In *H. hexastichum* awn, size, and fertility, of the lateral floret are controlled by a single factor. (ii) In some forms of *H. hexastichum* there may be present, in addition to this factor, a second, viz., that which in *H. intermedium* controls the size and fertility of the lateral floret. (iii) These lateral floret factors appear not to be linked with any other factor. (iv) The *H. hexastichum* lateral (factorially *AA* in the scheme of Harlan and Hayes) constitutes with the typical *H. distichum* lateral (*aa*) an allelomorphic pair.

## (2) *H. hexastichum* × *H. intermedium*.

The lateral floret characteristics of the parent forms are:

*H. hexastichum*—full awn : grains large : every floret fertile.

*H. intermedium*—no awn : grains small : fluctuable proportions of florets fertile.

These broad characteristics are commonly accepted in taxonomy and genetics. No data is available for the investigation of more detailed characteristics but that such exist is beyond doubt. Forms of *H. intermedium* occur in which the lateral florets never form large grains and never show a high proportion of fertility. Correspondingly there are forms characterised by grains almost as large as those of the laterals of *H. hexastichum* and with a high proportion of fertility. Possibly between these extremes there are definite intergrades. The work of Harlan and Hayes(7), alone bears definitely upon this matter. From this work and from the known inter-form differences, it seems certain that there must be one or more factors affecting fertility. No allowance can be made for these in the considerations involved here but it is to be borne in

mind that they may add to the complications which existing knowledge exposes.

Data upon this category of cross has been furnished by:

(a) Engledow(1) p. 104. A single factor was considered to control the awn, the size and the fertility of the lateral floret. Heterozygotes in  $F_2$  fluctuated widely [cf. (1) (d) above]. There was no evidence of linkage. To harmonise this result with the factorial scheme of Harlan and Hayes [*vide* (1) (e) above] the parents must be written  $AA.BB \times aa.BB$ . The *H. hexastichum* parent used was var. *pyramidatum*. Unfortunately this was not employed in the *H. hexastichum*  $\times$  *H. distichum* crosses so that the full test of conformity with Hayes and Harlan is not possible.

(b) Ikeno(4) has described a cross of awnless 6-row  $\times$  awned 6-row. It is believed that genetically the awnless parent should be regarded as *H. intermedium*. For that reason the evidence is considered in the present category of crosses. The proportion of fertile laterals in the awnless parent is very high and their size approaches that characteristic of *H. hexastichum*. The data appear to demonstrate clearly the segregation of one factor [*vide* Engledow(2) pp. 163-4]. But the proportion of the  $F_0$  types in  $F_2$  was about 1/64 and consequently a 3-factor explanation was essayed. Factor *A* was supposed to produce awns but of less than full length in both median and lateral florets: factor *E* had a precisely similar effect: *A* and *E* in combination produced full-length awns. An inhibitor *I* was held to suppress the awns of the lateral florets. For  $F_0$  the constitutions would thus be  $AA.EE.\dot{ii} \times aa.ee.II = H. hexastichum \times H. intermedium$ . The outstanding features of the theory are (i) In the lateral floret of *H. intermedium* awnlessness and fertility are distinct for *I* inhibits awn formation but is apparently without effect on fertility. (ii) To some extent median as well as lateral awns come under the control of *I*. To harmonise the actual data with the theory, assumptions are necessary, for example, that *II* completely suppresses the lateral awn but only partially suppresses the combination  $AA.EE$  which normally produces full-length awns in the medians.

The paucity of  $F_2$  data makes it impossible to examine critically the theory of this investigation or to compare it closely with the scheme of Harlan and Hayes which has so far helped to align the findings of different investigators. It is clear that the awnless parent employed will be of great service for further genetic analysis.

### (3) *H. hexastichum* $\times$ *H. decipiens*.

Upon this cross the observations of Biffen [(18) pp. 193-4] are very definite. The  $F_1$  plants resembled *H. distichum* and were staminate

while the  $F_2$  gave a 1:2:1 ratio for *H. hexastichum* :  $F_1$  type : *H. decipiens*.  $F_3$  growings confirmed this. Clearly, therefore, one factor governed fertility, size, and presence of awn in the lateral floret. This view is in harmony with the conclusions of (1) above, the form of *H. hexastichum* being, of course, *AA.bb*. A further pair of allelomorphs [cf. Conclusion (IV) of (1) (e) above] is thus constituted by the lateral floret forms of *H. hexastichum* and *H. decipiens*.

(4) **H. intermedium** × **H. distichum**.

From the evidence already recorded [Engledow(1) p. 105] and subsequent  $F_3$  and  $F_4$  evidence, it is concluded that perfectly simple unifactorial inheritance alone is involved. A third pair of lateral floret allelomorphs is thus established.

(5) **H. intermedium** × **H. decipiens**.

No evidence additional to that already referred to [Engledow(1) p. 105] is available. Unifactorial inheritance seems very probable but cannot be regarded as certain.

(6) **H. distichum** × **H. decipiens**.

From the data given by Biffen [(18) pp. 192-3] simple unifactorial inheritance seems to be clearly proved and a fourth pair of lateral floret allelomorphs demonstrated.

(7) **Inerme Crosses**.

The semi-*decipiens* form of the *inerme* lateral floret has been described in § V (*supra*). Awnlessness makes *inerme* very valuable genetically but its liability to fluctuation in both median awn and lateral floret is a great handicap. Crosses of other forms, however, are by no means free from similar difficulty. Indeed, every heterozygote, e.g.  $F_1$ 's of the crosses already discussed, seems peculiarly fluctuable in lateral floret form. Full details of *inerme* crosses are to be found in Engledow(2), § V above (*inerme* × *H. decipiens*), § VI above (Awn-Lateral Floret Linkage in *H. hexastichum* × *inerme*). For the crosses in turn, the most noteworthy features may thus be summarised :

(a) *H. hexastichum* × *inerme*.

With var. *parallelum* as the 6-row parent this cross gave no evidence of linkage. When var. *praecox* was used, however, a high linkage between full awns of medians and laterals, and fully fertile laterals (i.e. the 6-row habit) appeared to exist. The evidence, however (§ VI *supra*), was not completely satisfactory. That linkage is displayed when var. *praecox* is used in the cross but not when var. *parallelum* is used,

suggests that *Praecox* possesses a factor which *parallelum* lacks and which is linked with full awns. Such a factor, distinguishing different forms of *H. hexastichum* finds a place in the theory of Harlan and Hayes, viz. factor *B* which, actually, is the factor controlling the lateral floret form of *H. intermedium*. From what follows, however [(b) *infra*] it is clear that factor *B* is not linked with full awns. Thus the views concerning this linkage set forth in § VI (*supra*) do not accord with the theory which has proved to possess a general applicability in a number of other cases.

If, actually, there were no linkage, interpretation of results would be comparatively simple. Full awn and full fertility of the lateral floret of *H. hexastichum* would be attributable to a single factor. This would accord with the results of *H. hexastichum* × *H. distichum* crosses [*vide supra* Conclusion (i) of (1)] and would not necessarily militate against the general hypothesis of Hayes and Harlan.

(b) *H. intermedium* × *inerme*.

One form only of *H. intermedium*, was used in these crosses, viz., var. *Haxtoni* which has a lax rachis and whose glumes are distinctly smaller than those of *inerme*. This smaller type of glume behaves in inheritance exactly as if inseparable from the full awn of the median floret. In  $F_2$  fully-awned segregates had typically small glumes, heterozygotes had glumes of intermediate size, and awnless segregates had large glumes. The ratio of awn types in  $F_2$ —proved by growing a complete  $F_3$ —was full awn : half (fluctuable) awn : no awn = 23·83 : 50·72 : 25·45%. No other fact was forthcoming in support of the possibility of a linkage to which the small defect of full awn plants directs attention. In all three awn classes, lateral floret form showed simple unifactorial segregation. The following conclusions are therefore pointed:

(i) The awn of the median and the form and fertility of the lateral floret of *H. intermedium* are controlled by separate factors. These factors appear not to be linked.

(ii) *Intermedium* lateral and *inerme* lateral represent an allelomorph pair [cf. *supra* *H. intermedium* × *decipiens* in (5)].

(iii) Conclusion (i) represents a noteworthy contrast with *H. hexastichum* in which either the awn of the median is controlled by the same factor as the fertility of the lateral, or the two characters are controlled by separate but very highly linked factors.

(iv) Median awn is associated with smallness of glumes (of both median and lateral florets).

(c) *H. distichum*  $\times$  *inermis*.

The principal facts from the data already published (Engleadow(2) pp. 185-6] are:

(i) Large glume always accompanies the *inermis* lateral. The facts correspond precisely with those of (b) above.

(ii) Among the fully-awned segregates of  $F_2$ , laterals as small as those of *inermis* are never seen.

(iii) But in crosses with a dense eared form of *H. distichum*, differences in lateral floret size were observable among the full-awned  $F_2$  plants. Fertility was never attained by such laterals, though some of them were larger than the *H. distichum* parent: nor were any of them as small as the *inermis* type. In general, the denser the ear of the fully-awned segregate, the larger its lateral floret. Further data bearing upon this matter is contained in Appendix I.

(iv) When a lax eared form of *H. distichum* was used as parent, all the fully-awned segregates in  $F_2$  had lateral florets of about parental (*distichum*) size.

(v) In connection with (iii) and (iv) the following facts recorded by Harlan and Hayes [(7) p. 577] are of interest: "It will be seen that in the five crosses *H. hexastichum*  $\times$  *H. distichum* where the evidence is complete, the 2-rowed parents were dense-spiked. Aside from the fact that those which have been preserved came from such crosses, there is no evidence in the data now at hand that indicates inability to secure intermediates from crosses in which both parents are lax-spiked. The common varieties of lax 2-rowed barleys have less vigorous lateral florets than the common varieties of dense 2-rowed barleys. It is possible that the progeny of crosses where lax forms were used would be lower in fertility and the intermediates correspondingly less conspicuous. Hence, they would be less desirable and less likely to be retained as specimens." From the whole evidence available, incomplete as it is, the conclusion seems warranted that in *H. distichum* there are at least two distinctive forms of lateral floret. These, in some way, are associated with degrees of ear-density. The further prosecution of the lateral floret problem in *H. sativum* cannot, therefore, be separated from a study of ear-density, the foundations of which have been laid by Hayes and Harlan(8). Their results make clear that this necessary extension must greatly complicate the problem.

(vi) Awnless  $F_2$  segregates had *inermis* laterals save that on a few ears were one or two fertiles. As in similar cases previously discussed, these were regarded as fluctuants.



(vii) Half-awned  $F_2$  plants had laterals of variable size but in general they were of "inter-parental" form.

(viii) Median awn, typical *distichum* lateral, and small glume appeared jointly to be governed by one factor. This is an interesting contrast with conclusions (i) and (iv) of (b) *supra*.

(ix) The proportion of fully-awned plants appeared to be not less than 25 %. Neither this proportion nor any other fact suggested two highly linked factors instead of a single one.

(d) *H. decipiens*  $\times$  *inerme*.

The full detail of this cross has not been ascertained but two facts seem clear [*vide* § V *supra*].

(i) In  $F_2$  no partially-awned forms occur, all plants having either full awns as for *H. decipiens*, or no awns as for *inerme*. The ratio of these types = 1 : 3.

(ii) All fully-awned  $F_2$  plants have typical *decipiens* laterals. The remainder display three types of lateral, viz. *H. decipiens*, an intermediate form, and *inerme*.

It is difficult to form an appreciation of the considerable mass of detail which has been presented. Irreconcilable divergencies of view certainly exist, particularly in regard to the *H. hexastichum*  $\times$  *H. distichum* crosses. In part, these have sprung from differences of attitude and interest. The various lateral floret forms may be considered from the point of view of sexuality, e.g. *H. hexastichum* and *H. intermedium* are hermaphrodite, *H. distichum* staminate and *H. decipiens* sexless. Again fertility, size, and amount of awn, may be conjointly studied. Further progress will, it seems, necessitate attention to all the attributes of the lateral floret. It will demand, too, a far wider range of crosses. Harlan and Hayes have shown that two forms of *H. hexastichum* occur: ear-density in *H. distichum* is clearly associated with lateral floret form: in *H. intermedium* genetic degrees of fertility must be admitted: *Inerme* and awnless intermedium crosses give results out of harmony with those in which both parents have fully-awned medians. All these facts will need consideration in future work. From further *H. decipiens* crosses important indications are likely to come. In the past these crosses have been much neglected. If all the available evidence concerning "hoods" had been considered, a wider and still more difficult field would have been opened. For example, there are true breeding forms in which the hood is borne on a short awn. Forms of this kind will have to be genetically assessed before the whole case is made clear. In most inter-

group crosses of *H. sativum*, fluctuation is very marked. Consequently multifactorial theories like those of v. Ubisch and Ikeno are exceedingly difficult to test. A better understanding of lateral floret fluctuation may indeed be regarded as the most immediate requirement for the further investigation of the genetics of *H. sativum*. The number of forms of *H. sativum* scattered through the world must be very great. Many are clearly of natural-hybrid origin and a close systematic examination of all the forms which have been collected would materially aid genetic study. It would help to indicate the limitations to the possible combinations of the various attributes which are undoubtedly intimately connected—awn, grain size, fertility, glume size, and rachis density.

The genetic significance of the evidence of this paragraph is considered in that which follows.

#### VIII. MULTIPLE ALLELOMORPHISM IN *H. SATIVUM*.

In the first paper of this series [Engledow (1) p. 106] it was concluded that the four main groups of *H. sativum* were characterised by lateral floret forms which constituted a series of "multiple allelomorphs." The desirability of this phrase is sometimes questioned. In all recorded instances the factors of a multiple allelomorph series are similar, in that they control the same or strictly homologous parts of the organism. And further, their effects represent degrees: in other words, such factors constitute a quantitative series. From this point of view a multiple allelomorph series is not sufficiently remarkable to merit nomenclatural distinction. But Morgan and his collaborators have viewed the phenomenon differently. They consider the factors of such a series to represent an original and a succession of mutation factors, all of which occupy the same locus in the chromosome. Only two such factors can be present in an individual and linkages between members of the series and some other factor must all have the same value. This latter corollary is strongly supported by *Drosophila* data. If the only possible form of factor mutation is disappearance of the factor—according to some a necessary requirement of the Presence and Absence Theory—then multiple allelomorphism is a distinctive phenomenon with which the presence and absence view does not harmonise. Instead of postulating several mutants at the same locus it may be supposed that the factors of a multiple allelomorph series occupy adjoining loci. They are thus so close that cross-over among them is exceedingly rare—so rare as to escape notice. The arguments by which these views may be sup-

ported are familiar. Here, the purpose is not to assess the relative merits of these arguments but simply to present such facts from the data of § VII (*supra*) as bear upon multiple allelomorphism in its most general sense.

First the views of Hayes and Harlan [§ VII(1) (*e supra*)] may be re-considered for to a great extent they appear to be in general working agreement with a number of other results. Two types of *H. hexastichum* are believed to occur and in one of them lies the characteristic factor of *H. intermedium* thus :

$$\begin{array}{ll} H. \textit{hexastichum} \text{ 1st form} & = AA.BB. \\ \text{'' '' 2nd ''} & = AA.bb. \\ H. \textit{intermedium} & = aa.BB. \end{array}$$

These authors did not deal with other crosses such as *H. intermedium*  $\times$  *H. decipiens*, etc. so that they made no pronouncement concerning multiple allelomorphs. If, however, the four groups of *H. sativum* represent a multiple allelomorph series, and the genetic constitutions given above are to be accepted, it is clear that *A* and *B* cannot occupy the same locus. This might appear, in fact, to be a case of factors at adjoining loci: but such an arrangement could not fit the facts. For from the cross *H. hexastichum*  $\times$  *H. distichum* = *AA.BB*  $\times$  *aa.bb* the form *aa.BB* was obtained in  $F_2$ . For this, cross-over would be necessary and on the supposition of adjoining loci cross-over must be practically ruled out. It can only be concluded that the results here considered are not in accordance with the view that the lateral florets of the four main groups of *H. sativum* constitute a series of multiple allelomorphs.

Disregarding for the present the evidence from *inermis* crosses, the simplest results factorially are those of § VII(1) (*d*), (2) (*a*), and (3) (4) (5) and (6). They are all unifactorial results and confirm the view previously expressed [Engledow (1) p. 106] that the groups of *H. sativum* constitute a multiple allelomorph series. There is, of course, no circumstance which gives to these results a greater validity than to others. But where, after detailed investigation, divergent results are obtained, the simplest plan is to consider each view separately. Nothing in the evidence bears upon the different conceptions of the nature of multiple allelomorphs. Moreover, there is no reason to regard the four factors as anything but a simple quantitative series. In cases of this kind multiple allelomorphism is not a very noteworthy phenomenon. Morphologically, the quantitative series conception is acceptable. In *H. hexastichum* the lateral florets are perfect and nearly as large as the medians: in

*H. intermedium* they are perfect but smaller: in *H. distichum* they appear to be staminate only: in *H. decipiens* they are sexless. Mr. J. Line, of the School of Agriculture, Cambridge, is investigating the life-cycle of the four lateral floret forms. The work is not yet completed but so far the evidence closely supports the quantitative series view. In *H. intermedium* laterals the formation of spore mother cells is late compared with the median florets: in *H. distichum* somatic divisions cease just before the formation of the megaspore mother cell but pollen is formed: in *H. decipiens* somatic divisions cease very early and neither mega- nor microspore mother cell is formed.

The *inerme* crosses present great difficulties from the point of view of multiple allelomorphism as displayed in non-*inerme* crosses. They introduce the unique feature of the awnless median floret (*inerme* parent) and so make it necessary to consider median as well as lateral inheritance. The full results given in § VII(7) (*supra*) may be briefly re-stated in the following terms:

In *H. hexastichum* × *inerme* awned median and typical *H. hexastichum* lateral are very highly linked.

In *H. intermedium* × *inerme* awned median and typical *H. intermedium* lateral show no linkage.

In *H. distichum* × *inerme* awned median and typical *H. distichum* lateral are completely linked [or governed by only one factor.]

In *H. decipiens* × *inerme* awned median and *H. decipiens* lateral are inseparable (completely linked) and yet awnless median and *inerme* lateral are not linked. This cross differs from the other three in an additional particular. Heterozygous forms are completely awnless instead of half-awned.

Thus in *inerme* crosses the lateral florets of the four main groups of *H. sativum* show very different relationships. Such behaviour seems incompatible with membership of a simple quantitative (multiple allelomorph) series.

Ikeno's conclusions § VII(2) (*b*) upon an awned × awnless cross add to the difficulties already pointed out as does, perhaps, the evidence given at the end of § III (*supra*). From the whole evidence considered, it is clear that there are two great difficulties. Where different investigators have studied corresponding crosses, discordant results have often been obtained. In addition, the evidence from *inerme* crosses is both inconsistent with the view of simple multiple allelomorphism and, independently of this, difficult to interpret. But it has seemed desirable to discuss the difficulties in full for their insolubility has a distinct

genetic interest. A systematic re-investigation seems to be necessary. It would be a very considerable undertaking. The general principles of experiment noticed at the end of § VII (*supra*) appear to be desirable and, in addition, crosses might profitably be made among selected  $F_1$ 's. For example,  $F_1$  of *H. hexastichum*  $\times$  *H. decipiens* with  $F_1$  of *H. distichum*  $\times$  *H. decipiens*. Corresponding crosses involving *inermis* would also be valuable. From such experiments further knowledge might be gained concerning the multiple allelomorphism which, at first believed to be a simple phenomenon, appears from later evidence to be a complex one.

#### Appendix I. Some *H. distichum* $\times$ *H. distichum* Crosses.

The parents used were Chevallier, Archer, Plumage, Goldthorpe, Archplume, *H. spontaneum* (the reputed "wild barley"), and Spratt (usually classified as *H. s. zeocriton*). In the cases of each of the first four, three separate "forms" (i.e. individual selections) were used. Practically all the possible crosses of the parental forms in pairs were made. Complete  $F_2$ 's and partial  $F_2$ 's,  $F_4$ 's, and  $F_8$ 's were raised for most of the crosses. Plumage and Goldthorpe have a dense ear and, relatively to the lax-ear forms, rather expanded lateral florets. Archer has a lax ear, Chevallier being slightly laxer still. *H. spontaneum* has a fairly lax ear and its laterals are distinguished by the characteristic "triangular" termination of the outer palea. Spratt is very dense-eared and a difference in density between base and tip gives the outline of the ear a "truncated" form. Its laterals are distinctive, being rather long, slightly bent, and somewhat pointed at the tip.

It was hoped that this series of crosses, involving differences in ear-density and in lateral floret form—differences which in some cases appear to be associated—would cast some light upon the apparent minor variations of lateral floret form in *H. distichum*. [Cf. § VII(7) (c) in the text.] The closest examination of the  $F_2$ 's failed, however, to yield definite results. The gradations of lateral floret form were so fine that classification proved impossible. Ear-density very clearly segregated but its degrees, as judged by eye, could not be related to definite factorial schemes. The *H. spontaneum* crosses were the most baffling. With a few doubtful exceptions all the  $F_2$  plants appeared to display the outstanding characteristics of *H. spontaneum* itself—brittle rachis, very coarse awns and paleae, triangular pointed lateral florets. So marked was this phenomenon that at first accidental self-pollination instead of crossing was suspected where *H. spontaneum* was the  $F_0$  ♀.

But repetitions of the crosses confirmed the first result and, in addition, the  $F_1$  plants were always recognisably different from *H. spontaneum*. Some very curious genetic phenomenon must, it seems, underlie these crosses. Striking parallels, though a little less emphatic, are afforded by certain *Triticum* crosses in which considerable differences in ear-density distinguish the parents, e.g. Fife  $\times$  other forms of *T. vulgare*.

Inter-form lateral floret differences associated with degrees of ear-density in the various forms of *H. distichum* are suggested both by examination of these forms and by crosses of *H. distichum*  $\times$  *inermis*. The experience which has been recorded, however, suggests that their existence will not readily be demonstrated simply by making crosses. Factors which can modify lateral floret form even slightly, while not affecting fertility, demand attention. Their effects may possibly be responsible for some of the divergencies of result obtained by separate investigators working with similar but not identical crosses.

#### *Appendix II. A Case of Natural Cross Pollination in Barley.*

Natural cross-pollination is rare in wheat, barley, and oats. Practically all the recorded cases have occurred in warm climates. In England, the non-occurrence of the phenomenon is usually assumed in genetic work and experience has justified this statement. Nevertheless there exist in this country a small number of wheat forms whose origin must be attributed to natural crossing. Corresponding cases in barley appear not to be known unless, indeed, *H. intermedium* var. *Haxtoni* is such a one. [*Vide* Harlan and Hayes (7) pp. 575–6.] Rimpau, in a twenty-five year period observed, among his very numerous barley forms grown in Germany, only nineteen cases of natural out-pollination. Records of observed instances and a general résumé of evidence have been published by Pridham (20). Wheat is chiefly concerned and no barley example is mentioned for England. In some barleys the paleae gape noticeably at the time when the stigma is receptive and this, as Körnicke (6) pp. 137–40 pointed out, might be regarded as indicating imperfect cleistogamy. One of the most striking of these forms in England is *H. intermedium* var. *Haxtoni* but it has never been observed to become naturally cross-pollinated.

In view of the infrequency of the phenomenon in barley, the solitary instance which has been met appears to deserve notice. It occurred in a curious form named *thyrsoideum*. This is best described as a teratological hooded form of *H. hexastichum*. Irregular reduplication of the

florets occurs all along the rachis and results in a densely packed assemblage of florets of varying but always small size. In 1921 the plot of *thyrsoideum* was seen to contain two plants whose ears might be described as of hooded *H. distichum* form. At first they appeared to be rogues or mutants but close inspection of the lateral florets suggested that they were *H. spontaneum*  $\times$  *thyrsoideum* hybrids. These two forms, as the sowing plan showed, occupied adjoining plots in the previous year. From the seeds of the two plants the next generation was raised in 1922 and it left no doubt about the  $F_1$  nature of these plants. There were found many forms, e.g. *H. spontaneum* (awned, 2-row, brittle rachis, typical laterals), awned 2-row types with variable laterals, 6-row hooded, 2-row hooded, branching ears of the hooded 6-row type, branching ears of the awned 2-row type, *thyrsoideum*, etc. Quite clearly, in 1920, two *thyrsoideum* florets were fertilized by the pollen *H. spontaneum*.

In 1921 both *H. spontaneum* and *thyrsoideum* were again grown. The plots were in two separate "cages" and their distance apart was about fifty yards. The same natural cross-pollination occurred as in the previous year, for in the 1922 plot of *thyrsoideum* were five of the typical  $F_1$  plants. These two successive instances are noteworthy on account of the infrequency of the phenomenon among barleys in England and also, perhaps, from the fact that both involve the same parents.

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# NOTE ON KAMMERER'S EXPERIMENTS WITH *CIONA* CONCERNING THE INHERITANCE OF AN ACQUIRED CHARACTER.

By H. MUNRO FOX,

*Fellow of Gonville and Caius College, Cambridge.*

(With One Plate.)

IN *Nature*, Vol. cxi. p. 639, 1923, Kammerer, referring to his own investigations on the inheritance of acquired characters in salamanders and *Alytes*, wrote: "Not content with any of the previous experiments, I carried out, before 1914, what may really be an *experimentum crucis*." This concerned the inheritance of regenerated siphons in *Ciona intestinalis*. Kammerer states that when the siphons are cut off they regenerate longer than their normal length, repeated amputations giving very long tubes, and that the offspring of these operated individuals have siphons abnormally long.

I have repeated the amputation experiments and find that the regenerated siphons do *not* grow beyond the normal length. The work was done between June and September of this year at the Roscoff Marine Biological Station, and I wish to express my thanks to the Director for kindly putting at my disposition tanks on the walls of which numerous *C. intestinalis* were growing. In these aquarium-grown individuals the ratio of siphon length to body length is the same as in animals taken from the sea. The oral siphon was amputated in 102 individuals. The animals varied in length from .9 to 4.8 cm. As controls 235 unoperated individuals were kept under observation. In none of the operated animals, once the normal ratio of siphon length to body length had been reattained, was there any further abnormal growth of the siphons.

One operation was performed on 59 individuals, two on 35, and three on 8. In the animals operated upon more than once the siphons were allowed to regain their normal length before the second or third operation was made. The time necessary for the reattainment of the normal siphon length depended naturally on the level at which the cut had been made:

it varied from 14 to 44 days with an average of 27 days. The water temperature was 16.5°–19°. After the siphons had grown again to their normal length the animals were kept under observation sufficiently long to be certain that there was no further lengthening in proportion to the body-length. Individuals operated upon once were kept under observation from 22 to 61 days after the siphons had regenerated to the normal length, the average period being 42 days; those operated twice for an average of 34 days and those three times for 27 days after the last reattainment of the normal length of siphons. In no case did any abnormal growth of the siphons take place after the normal dimensions had been reached.

In addition to the above, the pharynx and atrium of a certain number of individuals were cut across so that both of the siphons were removed. Of these operated animals 14 lived to regenerate the amputated region but the new siphons never grew beyond the normal length.

Kammerer stated further (*loc. cit.*) that he removed the gonads from animals which had regenerated their cut siphons and that new gonads developed in place of those removed. The  $F_1$  generation bred from these new gonads, he said, showed the acquired character, so that here there could be no question of the siphon amputation having directly influenced the germ cells. Since, however, I found that the regenerated siphons did not grow abnormally long it was useless to repeat the operation of removing the gonads.

In 1913 it was shown at Naples that abnormally long siphons are developed by *C. intestinalis* when kept in suspensions of abundant algal food (*Biol. Centrbl.* Vol. xxxiv. p. 429, 1914). Plate I, fig. 1, shows a normal *C. intestinalis* and figs. 2 and 3 individuals with abnormally long siphons produced in this way. When animals which had thus developed long siphons were put back from the jar of abundant food into the aquarium circulation the original ratio of siphon length to body length was reattained by a relatively more rapid growth of the body as compared with the siphons. This is shown by Plate I, figs. 3 and 4. Further investigation proved that the abnormal siphon growth was due to the abundant food itself and not to the lack of motion of the water nor to its altered H-ion concentration, the other two factors by which the culture jars differed from the aquarium in which the normal animals were growing.

This mode of producing long siphons suggests that the abnormal growth in Kammerer's operated animals might have been due to their having been kept in jars containing abundant algal spores. Were this



1



2



3



4

Fig. 1. Typical *Ciona intestinalis* from aquarium tank ( $\times 1.5$ ).

Fig. 2. Similar individual to Fig. 1 nine days after having been placed in a jar containing abundant algal food ( $\times 1.4$ ).

Fig. 3. Similar individual to Fig. 1 thirteen days after having been placed in a jar containing abundant algal food ( $\times 1.7$ ). Immediately after being photographed this animal was put back in the aquarium tank.

Fig. 4. Same individual as Fig. 3 nine days after having been replaced in the aquarium ( $\times .75$ ).



the case, however, it should have been clear from controls of unoperated animals kept in the same water.

## ADDENDUM

A preliminary account of my experiments described above was published in *Nature*, Vol. cxii, p. 653, 1923. Since the MS. of the present Note was written several communications have appeared in *Nature* relating to these experiments. The most important are the following.

(1) Prof. E. W. MacBride (Vol. cxii, p. 759, 1923) writes that Dr Kammerer stated in a letter that when he cut off the oral siphon alone, leaving the anal siphon untouched, the oral siphon regenerated to a normal not to an abnormal length, "but when both anal and oral siphons were amputated in a very young animal, then long siphons are regenerated." I have recorded in the present Note that while in some individuals I amputated the oral siphon alone in others both siphons were cut off but in neither case were the regenerated siphons of abnormal length. Further, in a letter from Dr Kammerer which is in my possession it is stated that the removal of siphons with resulting hypertrophied regeneration can be successfully carried out with individuals of any size.

(2) Dr Kammerer points out (Vol. cxii, p. 826, 1923) that in 1891 Mingazzini at Naples obtained abnormal growth in the regeneration of amputated siphons of *Ciona intestinalis* (*Bol. Soc. Nat. Napoli*, anno 5, p 76). The result was the same when a single as when both siphons were cut. Mingazzini does not state however whether the operated animals were kept in the aquarium circulation or in other vessels, in which the quantity of food might have differed. But if his observations are correct *Ciona intestinalis* must behave differently at Naples and at Roscoff. Kammerer's work (according to his private letter referred to above) was done at Vienna with animals and water from Trieste.



# RUDIMENTARY PARTHENOGENESIS IN *TENEBRIO MOLITOR* L.

By DR A. M. FREDERIKSE.

(With one Plate and a Text-figure.)

## INTRODUCTION.

MANY experiments with artificial parthenogenesis have been carried out and many cases of parthenogenesis as a normal phenomenon have been studied, but we know little of the fate of unfertilized egg-cells of those animals in which impregnation of the egg by the sperm is necessary, or at least the rule, in reproduction. For a general review of all that is known about this subject up to 1910, we cannot do better than study the paper of M. A. Lécaillon, "La Parthénogénèse naturelle rudimentaire<sup>1</sup>."

Lécaillon also published a brilliant paper, "La parthénogénèse chez les oiseaux<sup>2</sup>." Lécaillon conducted researches, both macroscopical and microscopical, on the unfertilized eggs of the fowl and the peahen, after keeping these eggs at the temperature of the room or incubating them. He treats the question in full detail and gives many facts which are in accordance with our investigations on the unfertilized egg of *Tenebrio molitor* and therefore perhaps of general occurrence even in animals belonging to very different groups.

His results may be summarized as follows. There is a beginning of development, blastomeres are seen in the germinal disc, and all around this groups of nuclei are found scattered in that part of the plasma, in which no cleavage is observed. These groups are called by Lécaillon "*nids de noyaux*." Many of the cell processes show abnormal behaviour. Lécaillon describes pluripolar mitotic figures, cells with many nuclei, abnormal chromosomes, etc.

In Coleoptera rudimentary parthenogenesis has been studied only in two cases: a short note of Saling, "Notizen über Parthenogenese bei *Tenebrio molitor* L.<sup>3</sup>," and one of Osborne on parthenogenesis in *Gastrophysa raphani*<sup>4</sup>. In the youngest egg Saling describes the formation of

<sup>1</sup> Bull. Sc. France et Belg. T. XLIV. 1910.

<sup>2</sup> Arch. d'Anat. microsc. T. XII. 1909.

<sup>3</sup> Zool. Anzeig. Bd. XXIX.

<sup>4</sup> Nature, Vol. XX. 1879, Vol. XXXI. 1880.



a polar body (by no means a clear description as it is uncertain if it was the first or the second one). He states an increase in the number of nuclei, which are descendants of the maternal pronucleus. These nuclei are spread in the deutoplasm. Besides these typical nuclei Saling describes small plasma areas in which he observes chromatic substances in the shape of rings and fragments, regarding them as degenerating descendants of the maternal pronucleus. They are finally resorbed.

Osborne did not study the non-fertilized eggs of *Gastrophysa raphani* microscopically, but gave only an account of the facts he observed with the naked eye. In 1879 he observed one case of development of an unfertilized egg out of the 900 eggs laid by a virgin female. Various parts of the animal, as legs, mandibles and antennae could be detected; also slight movements of these parts were observed; the embryo however died. The next year, the percentage of development was much greater, and one full grown individual (female) was obtained which laid a batch of fertilized eggs after copulation.

#### TECHNIQUE.

The greatest difficulty I met with in studying the unfertilized eggs of *Tenebrio molitor* was the fixation and the cutting of the eggs. The hard chitinous coat and the great bulk of deutoplasm were the causes of these difficulties. Different methods of fixation were tried, such as mixtures containing osmic acid, sublimate (Petrunkewitsch), chromates, chromic acid (Zenker), Bouin's fluid, Bouin with urea and formaldehyde solutions. The best result was obtained by boiling the eggs for three minutes in 1:3 formalin solution, containing nearly 10 per cent. formaldehyde; directly after this the eggs were plunged into 40 per cent. alcohol. Nitric acid-alcohol-sublimate and also Petrunkewitsch's fluid gave a good fixation of the egg as a whole, but the contents of the cells were always difficult to stain. I will return to this later on.

The eggs which I studied were collected from a population of virgin females, which were kept in the incubator at a temperature of 24–30° C. The eggs were laid on woollen threads or on buckwheat husks. The beetles were fed with little bits of raw potatoes and rusks, as described by Arendsen Hein ("Technical experiences in the breeding of *Tenebrio molitor*")<sup>1</sup>. Fixation of the eggs took place one to five days after they had been laid.

<sup>1</sup> *Proc. Konink. Akad. v. Wetensch. Amsterdam*, Vol. XXXIII. No. 1.

## OBSERVATIONS.

The first thing I noticed was that the degree of development of eggs of the same age varied much in different collections. Sometimes the eggs of one lot showed no or hardly any signs of development, whilst others belonging to another lot all showed a much more advanced state of development. When development was observed, it was always much retarded in comparison with the normal development of a fertilized egg. This is also mentioned by Lécaillon in the fowl and by various authors in *Serica mori*.

The youngest egg I found was at the moment when the first polar body was formed. It is impossible to speak of a mature egg yet. The next oldest egg was forming the second polar body. It is often stated that the formation of the second polar body takes place only immediately after fertilization, or at the same time. This was certainly not the case here. Moreover, the fact that without fertilization the second polar body is expelled from the egg, is different from what happens in those eggs in which, with normal parthenogenesis, no second polar body is formed. That in this case it is really the second polar body is beyond any doubt, as the first polar body is seen outside the egg and the female nucleus already parted in two, in forming the second polar body (Plate II, figs. 1 and 2). This doubtful point in Saling's note (see also Lécaillon, *Bull. Sc. F. et B. t.* XLIV. p. 252) is thus cleared up.

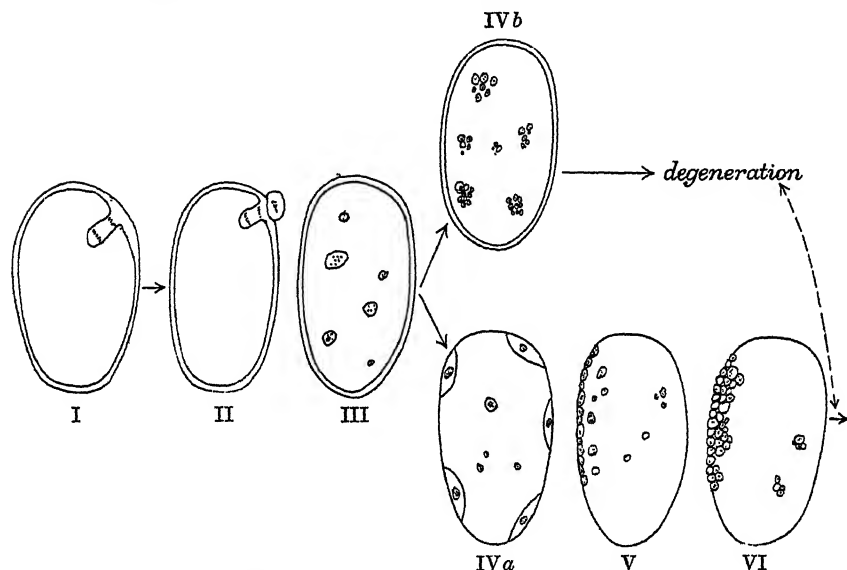
Figs. 1 and 2 are also important for establishing the number of chromosomes. In this case of the formation of polar bodies, however, this number cannot be ascertained with certainty. It is very likely that it will prove to be the haploid number, i.e. 10, but it may be lower, as the figure shows. The haploid number would be in accordance with what Tafani found in his investigation of the unfertilized egg cell of the mouse.

The next stages in development behave like the normal fertilized egg. After the multiplication of the maternal nucleus, the nuclei formed spread in the yolk (Scheme, fig. III). After this the development may go in different directions. It may happen that there are formed groups of nuclei at different parts of the egg, each group lying in a small area of plasma in the yolk (Scheme, fig. IV *b*). Or it may happen that this formation of groups of nuclei is inconsiderable or of no importance and that only a few large cells appear at the surface of the egg (Scheme, fig. IV *a*), each embedded in a small territory of plasm.

It is a remarkable fact that the above-mentioned differences may

## 96 *Rudimentary Parthenogenesis in Tenebrio molitor L.*

regularly be observed in eggs belonging to lots collected at different times. The eggs belonging to one lot have the same mode of development, the different lots differ from each other. In the eggs of one collection we may not find these isolated cells at the surface of the egg, but only groups of nuclei in areas of protoplasm, here and there distributed in the yolk, whilst in nearly all the eggs of another collection there are not many nuclei spread in the yolk, but only a few large isolated cells are found all lying at the surface of the egg (Scheme, fig. IV a). These



Scheme illustrating degenerative process.

differences may be due to individual differences in the beetles themselves (one beetle depositing eggs having a greater developmental power than another), or they are caused by conditions of environment (i.e. changes in the humidity of the atmosphere, in feeding, in temperature, i.e. from  $\pm 24^{\circ}\text{C.}$ – $30^{\circ}\text{C.}$ , etc.).

It is not probable that the former possibility (individual differences) plays a great part in this, as from a given population it may be expected that at a given time all the eggs have, on the average, the same developmental power. It is true that the composition of the population did not remain constant. The number of its individuals diminished by the death of some and was increased by new comers. But this change is only a gradual one for the population as a whole. It is therefore probable that the variable environmental conditions for a great part and the individual

differences for a small part bring about the above-stated variable results. Regarding this matter, I may refer here to one of the conclusions of Osborne, viz. "...that it [developmental power of unfertilized eggs] is peculiar to some females, while others appear to be exempt from it."

In eggs of about four days old, a much greater augmentation of the spread groups of nuclei in the areas of plasm is noticed. Sometimes a smaller or greater part of the egg surface is covered with cells more or less irregular in form and distribution. In these cells distinct mitoses are often seen (Plate II, figs. 3 and 4). It is therefore certain that formation of blastomeres has taken place. I never saw these blastomeres extended on the whole surface of the egg.

The irregularity of the blastomeric formation is not limited to its distribution on the egg surface; it is also irregular when it appears at different places in more layers. At first there is only one layer of cells more or less close together, whilst often at a little distance under this layer a few spread cells between the yolk are found (Plate II, fig. 3). These cells divide and form a second layer against the first. These observations are not in accordance with those stated by Saling, who says, "zu einer Blastodermbildung kommt es aber keineswegs..." (S. 588).

How can we explain the difference in the observations of Saling and mine? Saling fixed his eggs in nitric acid-sublimate-alcohol before studying them, and mentions nothing of the use of another method of fixation. Now I have already stated above, that by this method of fixation the contents of the nucleus are not clearly shown and therefore the blastomeres are not distinctly visible. So it is possible that Saling has not studied a great number of unfertilized eggs and may not have seen eggs in which the formation of blastomeres occurred.

Beyond an irregular distribution of the blastomeres in the unfertilized egg of *Tenebrio*, these blastomeres cover only a part of the egg, whilst in the fertilized egg of *Tenebrio* there is one layer of cells lying close together and covering the whole surface of the egg (see Plate II, figs. 4 and 5); there are also irregularities seen in the cells and nuclei themselves. At the moment of the formation of the equatorial plane, it is in the fertilized egg, as a rule, easy to state that the chromatic elements in the blastomeres lie close together, so that viewed from the side (not from the poles), the chromosomes form together a stripe of very small breadth.

This is never the case in the unfertilized eggs where the chromosomes are spread in a greater area, so that the chromosomes are seen most often apart from each other. Whilst in the blastomeres of the fertilized eggs the chromatic elements are nearly the same and all the nuclei seem

to possess the same chromatic contents; this is not the case in the blastomeres of the unfertilized eggs. The amount of chromatic elements in the blastomeres varies in a high degree; also the shape of the chromosomes, which present themselves in the form of threads and little bars, which are not the usual form of chromosomes in *Tenebrio*. It is obviously a direct consequence of the fluctuation in chromatic contents that the size of the blastomeres fluctuates also. In the large blastomeres we see always a much greater amount of chromatic substance than in the other blastomeres (see Plate II, figs. 3, 6 and 10).

I have not succeeded in staining the irregular mitotic figures in the same magnificent way as Lécaillon did in the case of the fowl, notwithstanding my many attempts in testing the different fixation fluids already mentioned.

Besides the irregular distribution of the chromatin in the two halves of the mitotic figure, and the deviations in the form of the chromosomes, I have now and then also noticed irregularities in other parts of the dividing mechanism, as may be seen in Plate II, figs. 6, 8, 9 and 11, in which anomalies in the achromatic figure are shown. In Plate II, fig. 9 we see a cell with four nuclei and in the midst of it a centrosphere. Plate II, fig. 6 gives a hyperchromatic nucleus, at the beginning of mitosis, with three centrospheres around it. The very different amounts of chromatin in the two parts of the dividing nucleus are plainly visible in Plate II, figs. 3 and 7; so are the anomalous forms of chromosomes. This is in full accordance with the results mentioned by Lécaillon for the fowl and by Bataillon for *Petromyzon Planeri*. I have never seen unfertilized eggs of *Tenebrio* further developed than the stage of irregular blastomeric formation described.

In the oldest eggs described by Saling (five days), he distinguishes in the yolk plasm many chromatin particles from the descendants of the maternal pronucleus and plasma areas with loose meshes in globular form with condensed small rand, which stains intensely; these areas contain yolk elements which are fused together.

That these eggs at the age of five days show degenerating regions is most probable. I believe that Saling sets too much value upon this phenomenon and therefore forgets the facts of the divisions and augmentation of the nuclei which are spread all around in the yolk. It is not always easy to state whether we have to do with a yolk nucleus (*vitellophage*) or a degenerating plasm particle. That the first supposition is of frequent occurrence is to be seen in much younger eggs, fertilized or not, where the same things are noticed (Plate II, figs. 13 and 14). In eggs where

blastomeric formation occurs, with mitotic figures in the blastomeres, it is not reasonable to think there will be so many foci of degeneration in different parts of the egg. When this mode of origin described by Saling was of frequent occurrence, intermediate conditions must be found between the forms described by Saling and the deutoplasm granules; this however does not occur. These forms are dissolved in 5 per cent.  $\text{NH}_3$  solution, just as chromatin, which may be an indication of their origin. It is interesting to observe that Lécaillon gives a figure of the same things (see fig. 56, *Arch. d'Anat. microsc.* T. XII). They appear in the fowl in the same way, as in the unsegmented part of the egg of *Tenebrio*.

Neither Lécaillon nor I could ever detect a mitosis in these particles. They may grow out to great complexes (Plate II, figs. 13 and 14). How do they increase in number? Lécaillon ("*nids de noyaux*") thinks they multiply by budding and amitotic division. In such a case where there is augmentation in number of nuclei without mitotic figures, there are always forms of nuclei which can be explained as having come into existence by budding or amitotic division. We can accept such an explanation until we have found the right way to trace the mode of their origin.

#### SUMMARY.

It is evident that in animals belonging to the most different groups, the unfertilized eggs have more or less the power to develop. This is therefore a common property of the egg cell. Control experiments are therefore in experimental parthenogenesis a necessity. The development takes place very slowly and is irregular. The development frequently stops early, after which degeneration sets in. The mitosis is often irregular. There is perhaps budding and amitosis.

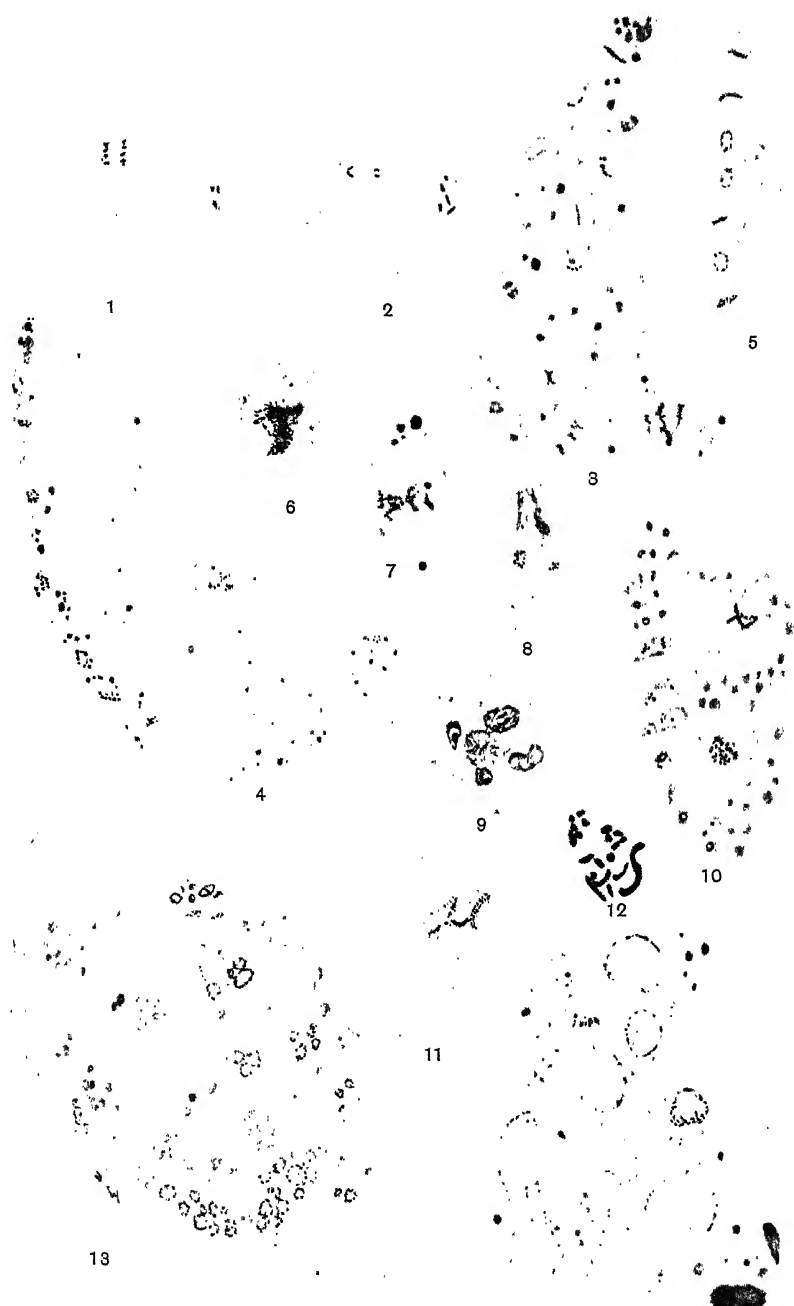
The virgin females I used in this research were sent me by Mr Arendsen Hein, Utrecht; I wish to thank him in this place. As the females were separated in the pupal state, the possibility of fertilization was absolutely excluded.

ZUIDWOLDE (DRENTHE), HOLLAND.

July 5, 1923.

## EXPLANATION OF PLATE II.

- Figs. 1 and 2. Forming of the second polar body in unfertilized egg of *Tenebrio molitor* (from two successive sections).
- Fig. 3. Part of unfertilized egg, showing irregular blastomeric formation. Leitz, obj. 6, oc. 3.
- Fig. 4. The same. Leitz, obj. 4, oc. 3.
- Fig. 5. The same of fertilized egg of *Tenebrio*. Not much older than one day.
- Fig. 6. Irregular hyperchromatic mitose in unfertilized egg, showing three centrospheres.
- Fig. 7. The same. Note the irregular form of the chromosomes.
- Fig. 8. Irregular mitotic figure.
- Figs. 9 and 11. Irregular pluripolar mitotic figure.
- Fig. 10. Abnormal great cells, under irregular blastomeric formation in unfertilized egg.
- Fig. 12. Abnormal chromosome forms in blastomeric cells.
- Fig. 13. Degenerating egg, 5 to 6 days, obj. 4, oc. 3.
- Fig. 14. "*Nids de noyaux*," obj. 6, oc. 3.







# FURTHER STUDIES ON INHERITANCE IN *MATTHIOLA INCANA*.

## I. SAP COLOUR AND SURFACE CHARACTER.

By EDITH R. SAUNDERS,

*Fellow of Newnham College, Cambridge.*

### 1. *Introduction.*

SUCCESSIVE contributions to the study of the inheritance of surface character, sap colour, plastid colour and doubleness in the Garden Stock (*Matthiola incana*) have appeared since 1902 (Saunders, 1-11), in which the conclusions gradually arrived at in respect of these characters have been formulated and expressed in terms of factors. These expressions give the expectation, realised again and again in innumerable experiments, for a great variety of unions, and as to the correctness of the main generalisations there can be no doubt. But further work has made apparent the existence of other factors and of additional interfactorial and gametic relationships which are not exhibited in these expressions as they stand. Before entering upon any fresh consideration of the factorial scheme, however, it will be advisable to review, in so far as they concern the present discussion, the fundamental facts and conclusions upon which the scheme is based. Briefly stated, they are as follows:

1. Certain white-flowered strains of different and appropriate constitution, when crossed together, constantly give coloured offspring (of some blue or purple shade if a third factor *B* is present, otherwise of some shade of red). Hence it is clear that sap colour in the petals must be due to (at least) two factors (indicated by *C* and *R*), in the absence of either or both of which the sap is always uncoloured.

2. Certain glabrous strains of different and appropriate constitution, when intercrossed, constantly give fully hoary offspring. Since this result obtains with sap-coloured (as well as with non-sap-coloured) forms, full hoariness must be due to (at least) two factors (indicated by *H* and *K*), which are, however, only effective when *C* and *R* are also present. (If the allelomorph *H*<sub>1</sub> is present in place of *H* the plant exhibits only the degree of hoariness termed half-hoary.)

## 102 *Further Studies on Inheritance in Matthiola incana*

3. Certain single-flowered strains (pure-breeding) produce exclusively single-flowered offspring, while others (eversporting) yield in every generation a mixture of singles and doubles in which the doubles slightly preponderate.

4. The ratio of singles and doubles obtained from the eversporting forms, probably 15 singles : 17 doubles, taken in conjunction with the fact that the pollen of these strains has been found to be all of one kind and to carry only doubleness, while the ovules carry some doubleness and some singleness, is explicable on the supposition that singleness is due to (at least) two factors (indicated by  $X$  and  $Y$ ), which segregate separately but not wholly independently, the gametic ratio for the ovules of the eversporting class being probably

$$\begin{array}{l} 15 \text{ } XY \\ 1 \text{ } Xy \\ 1 \text{ } xY \\ 15 \text{ } xy \end{array}$$

while the pollen will all be represented by  $xy$ .

5. The results obtained in  $F_2$  from matings between pure-breeding and eversporting singles are explicable on the supposition that the  $XY$  factors are linked together (indicated thus,  $\widehat{XY}$ ) in the pure-bred true-breeding single strains, so that they do not segregate separately in  $F_1$  cross-breds derived from matings between these two classes of singles.

6. Colourlessness in the plastids behaves as a dominant to cream and is due to the presence of a single factor (indicated by  $W$ ), which, in the peculiar eversporting sulphur-white (which is heterozygous for plastid colour as well as doubleness) is linked to one of the  $XY$  factors (taken to be  $X$ , and indicated thus  $\widehat{WXY}$ ). Hence the gametic ratio for the ovules of this strain is

$$\begin{array}{l} 15 \text{ } \widehat{WXY} \\ 1 \text{ } \widehat{WXy} \\ 1 \text{ } wxY \\ 15 \text{ } wxy \end{array}$$

while the pollen, which here is all cream as well as all double, is represented by  $wxy$ .

The relationships set forth above sufficed to account for the results observed with some outstanding exceptions. In these particular matings the marked deficiency of doubles recorded in several of the  $F_2$  families seemed to point to some further complexity. These cases occurred in

unions in which pure-breeding singles were crossed with an eversporting strain, and it appeared possible that a second pair of factors producing singleness (indicated by  $X'$  and  $Y'$ ) might here be concerned as well as  $X$  and  $Y^1$ , but further testing of this suggestion was needed. In fact a more extended investigation in general of the gametic ratios in  $F_1$  cross-breeds heterozygous for the  $WXY$  factors was desirable, which should at the same time either confirm or disprove the method suggested for reconstructing an eversporting individual from the descendant of a mating between eversporting and pure-breeding singles which was itself no longer eversporting<sup>2</sup>. Finally the fact that only a very limited number of the possible factorial combinations of the  $CRHK$  factor group were to be found among the strains on the market rendered it almost necessary to make and stock the unobtainable combinations so that the complete series might be available for testing purposes. It was with these immediate objects in view that further breeding has been carried on. Necessarily a lengthy undertaking in any case, the work has been further prolonged owing to the carrying out coincidentally of a full investigation of the relationships of the half-hoary form (*semi-incana*), a type which had been utilised in some of the earliest experiments, but the relationships of which to some of the other types employed had not at the time been fully worked out. Now, however, a stage has been reached in the direction of fulfilment of some of these aims which calls for a certain re-statement of the original scheme. For whereas in the earlier work indication of linkage between the factors  $C$ ,  $R$ ,  $H$  and  $K$  had not been observed, it has since become clear that the members of this group do not in all cases segregate wholly independently. It is now moreover evident that the mode of procedure outlined in the earlier account for synthesising an eversporting from a non-eversporting individual needs modification. The original suggestion<sup>3</sup> had been based on the supposition that the distribution of the involved factors among the gametes of the  $F_1$  cross-bred from the preliminary mating  $\widehat{W}XY \times w\widehat{X}\widehat{Y}$  would be the same for ovules and pollen. This, however, now turns out not to be the case. Such  $F_1$  cross-breeds, when selfed, are found to give equal numbers of whites and creams, a result due to the fact that  $W$  is not borne by the pollen<sup>4</sup>. Hence the necessity for modification of the method to be pursued in accord with this new fact. It is proposed, however, to treat fully the relations existing between

<sup>1</sup> *Journ. Genetics*, Vol. I. No. 4, pp. 338, 341, 358 and Table IV, 1911; *Comp. Rend.* p. 402, 1913 (IV<sup>e</sup> Conférence internationale de Génétique, 1911).

<sup>2</sup> *American Naturalist*, Vol. L. p. 496, 1916.

<sup>3</sup> *Ibid.*

<sup>4</sup> Or if it is so borne, it must be but rarely.

singleness and doubleness and plastid colour in cross-breds and their derivatives in a separate communication, and to deal in the present account only with the factor group affecting sap colour and surface character.

2. *The inter-relationships of the factor group determining sap colour and hoariness.*

As stated above, no indication of linkage between these four factors had been detected in the earlier results. But records obtained in the attempt to identify among the descendants of certain matings those factorial combinations not obtainable on the market led to the belief that where equality of the various combinations might be looked for, some were in fact present in the expected abundance, but that others were scarce, if not absent altogether. In addition to the available commercial combinations, viz. *CRHK*, *CRH*, *CRK*, *RHK*, *CK*, and (?) *CHK*, the missing forms *CR*, *C*, *K*, 0 (*crhk*) were obtained from appropriate cross-breedings, identified and stocked. But the failure to find the complementary forms *R* and *H* suggested that in some strains of individuals these factors must be either partially or completely linked together. This conclusion has been confirmed by the fact that in a number of cases in which  $F_1$  cross-breds from the matings  $CK \times RHK$  and  $CRH \times CK$  or their reciprocals have been self-fertilised or crossed with another strain of known composition, the  $F_2$  ratios of hoary and glabrous have been those which we should expect if the factorial difference between the parents had been of a lower order than it actually was. Thus, where all four factors were combined in the  $F_1$  cross-bred, the parents containing only *one* out of the four in common, the ratio actually recorded was what we should expect if the parents had contained *two* common factors, supposing all of them to be unlinked. Similarly, where the parents had in fact two factors in common, the ratio of hoary to smooth agreed with the expectation for a case where *three* unlinked factors are common to both parents. Again, where the anticipation from a back cross was  $7H : 1G$  and  $3H : 1G$  the ratio obtained was sometimes  $3H : 1G$  and  $1H : 1G$  respectively. That is to say, where the expectation if all four factors segregated independently was  $81H : 175G$ , the numbers recorded in some cases pointed to a ratio of  $27H : 37G$ ; similarly when the expectation was  $27H : 37G$  the ratio obtained was  $9H : 7G$ ; when  $9 : 7$  was expected  $3 : 1$  was recorded<sup>1</sup>. It was evident from these results

<sup>1</sup> See *Proc. Roy. Soc. B*, Vol. LXXV. p. 545, 1912. Also *Journ. Genetics*, Vol. v. No. 3, p. 149, 1916.

that two members of the  $CRHK$  factor group must, in such cases, be linked, and be segregating as a single unit after the manner of  $XY$  in the pure-breeding singles. Reference to the results set out in Table I ( $F_1$  + self) and Table II ( $F_1$  + various types and cross-breeds) will make it clear that in every union linkage was general. Only in the few families marked with an asterisk was the record in agreement with expectation based upon simple factorial difference.

The fact that  $C$  without  $K$ , and  $K$  without  $C$ , occurred among the offspring from a mating between two extracted  $F_2$  individuals of which one contained  $CRH$  and the other  $K$ , while no individual was discovered which contained  $R$  without  $H$ , or  $H$  without  $R$ <sup>1</sup>, pointed to linkage between  $R$  and  $H$ , and this has now been established for the various commercial sap-coloured strains containing  $CRH$ , and non-sap-coloured creams and sulphur-whites containing  $RHK$ , which consequently should henceforth be represented as  $CR\widehat{H}$  and  $R\widehat{H}K$  respectively. This fact explains the puzzling circumstance that up to the present all attempts to recover by suitable matings the (?) lost form  $CHK$  have proved unsuccessful.  $CHK$  whites (or so it was believed) were used in some of the earliest experiments, at a time when the possibility of factorial linkage had not yet been entertained. Now that it is realised that the  $F_2$  ratio of 9 hoary to 7 smooth may indicate  $R\widehat{H}K \times CK$  and not  $RHK \times CHK$ , the indirect evidence furnished by this ratio alone does not permit us to assume the presence of  $H$  in the  $CK$  parent, as had originally been thought to be justified. [That the original whites employed were not *directly* tested for  $H$  before they were lost arose from the fact that of the two forms which would have served this purpose, one—the  $CRK$  strain—had not then been met with in the commercial material obtained, while the other—the half-hoary type ( $CRH_1K$ )—happened to belong to the class of intermediate Stocks, and unless sown in the preceding autumn was not available at the time that the Ten Week strains were in flower. It fell out in consequence that the relations of this type to the various glabrous forms was not fully investigated until several years later.] Neither does a record of the  $F_2$  flower colour provide a sure means of distinguishing between these two matings, for in *both* unions all the  $F_2$  hoary will be sap-coloured and all the glabrous non-sap-coloured. As at no later date, however, has any  $CK$  white or sulphur-white, when crossed with *semi-incana* ( $CRH_1K$ ) ever yielded a full hoary  $F_1$ , as it should do did it

<sup>1</sup> See later, p. 110. Nevertheless individuals so constituted do presumably occur, see p. 111.

106 *Further Studies on Inheritance in Matthiola incana*

TABLE I. *Analysis of  $F_2$  families derived from self-fertilisation of  $F_1$  cross-breds.*

\* indicates a family in which  $R$  and  $H$  appear *not* to be linked.

‡ signifies that the numbers recorded cannot be taken to be decisive.

† is placed against those families which have been recorded in an earlier account but which are included here in order to present the whole of the evidence together.

ext. (=extracted) indicates an individual of crossbred descent.

sulph. = sulphur-white.

Expectation if  $R$  and  $H$  are unlinked ( $RH$ ) =  $27H : 37G$ , if  $R$  and  $H$  are linked ( $\widehat{RH}$ ) =  $9H : 7G$ .

Exp.	Reference numbers		Form of mating and factorial constitution of parents (Linkage of $R$ and $H$ not shown)	$F_2$	
	Family	Parents		Hoary	Glabrous
1	1	(13801 $\times$ 1383) $\times$ self	(sulph. $B \times$ sulph. $A$ ) $\times$ self ( $CK \times RHK$ )	28	21
	2			47	27
	3			26	18
	4			15	9
	5			31	22
	6			34	22
	†*7			43	54
	8	(13803 $\times$ 1383) $\times$ self		25	20
	9			89	53
Of the 246 glabrous plants 201 were flowered. None had coloured sap.					
2	1	(5223 $\times$ 18137) $\times$ self	(sulph. $B \times$ d. cream) $\times$ self $CK \times RHK$	64	48
	2			98	77
	3			35	28
	4			71	60
	5			20	21
	6			20	13
	7			29	28
	8	(5224 $\times$ 18137) $\times$ self		27	26
	9			77	51
	10	(5228 $\times$ 18137) $\times$ self		44	30
	11			22	14
	12			46	39
	13			40	34
	14			52	44
	15	(5234 $\times$ 18137) $\times$ self		35	27
	16			59	47
	17			102	73
	18	(5229 $\times$ 18144) $\times$ self		34	29
	19	(5236 $\times$ 18144) $\times$ self		140	129
	20			88	66
Of the 884 glabrous plants 407 were flowered. None had coloured sap.					
3	†*1	(44163 $\times$ 18233) $\times$ self	(sulph. $B \times$ no-d. cream) $\times$ self probably ext. ( $CK \times RHK$ )	33	50
	2			45	42
	3			62	44
	4			54	35
	5			148	122
	6			12	8
	7			13	6
	*8			8	13
Of the 380 glabrous plants 172 were flowered. None had coloured sap					

TABLE I (*continued*).

Reference numbers		Form of mating and factorial constitution of parents (Linkage of <i>R</i> and <i>H</i> not shown)	<i>F</i> <sub>2</sub>	
Exp.	Family	Parents	Hoary	Glabrous
4	†*1	(1342 × 31156) × self Of the 24 glabrous plants: 9 were not recorded 9 were sap-coloured 6 were non-sap-coloured	18	24
5	1	(1887 × 1338) × self (no-d. white × sulph. <i>A</i> ) × self ( <i>CK</i> × <i>RHK</i> ) Of the 63 glabrous plants 53 were flowered. None had coloured sap.	78	63
6	1	(1554 × 1954) × self (no-d. red × sulph. <i>B</i> ) × self ( <i>CRH</i> × <i>CK</i> ) Of the 123 glabrous plants: 42 were sap-coloured with stigmatic and hydathode hairs 15 also containing <i>CRH</i> and bearing an occasional hydathode hair nevertheless did not become coloured 66 bore no hairs and were non-sap-coloured	141	123
7	1	(833 × 1342) × self (d. flesh × sulph. <i>B</i> ) × self ( <i>CRH</i> × <i>CK</i> ) Of the 32 glabrous plants: 12 were not flowered 4 were sap-coloured 16 were non-sap-coloured	42	32
Expectation if <i>R</i> and <i>H</i> are unlinked ( <i>RH</i> ) = 81 <i>H</i> : 175 <i>G</i> , if <i>R</i> and <i>H</i> are linked ( <i>RH</i> ) = 27 <i>H</i> : 37 <i>G</i> .				
8	1	(41102 × 4131) × self (ext. no-d. white × ext. no-d. white) × self (ext. <i>RHK</i> × ext. <i>C</i> ) Of the 15 glabrous plants: 1 was not recorded 2 were sap-coloured 12 were non-sap-coloured	11	15
Expectation if <i>R</i> and <i>H</i> are unlinked ( <i>RH</i> ) = either 81 <i>H</i> : 175 <i>G</i> or 27 <i>H</i> : 37 <i>G</i> , if <i>R</i> and <i>H</i> are linked ( <i>RH</i> ) = either 27 <i>H</i> : 37 <i>G</i> or 9 <i>H</i> : 7 <i>G</i> according as <i>F</i> <sub>1</sub> = <i>CRHCK</i> or <i>CRHcK</i> .				
9	†*1 †*2	(195442 × 2349) × self (ext. no-d. purple × ext. no-d. white) × self (ext. <i>CRH</i> × ext. <i>CcKK</i> )	17 67 66 89	33 135 58 83

In all four families sap-coloured and non-sap-coloured glabrous individuals occurred but only a few were flowered.



# 108 *Further Studies on Inheritance in Matthiola incana*

TABLE II. *Analysis of  $F_2$  families derived from cross-fertilisation of  $F_1$  cross-breds.*

Signs and abbreviations as in Table I.

Expectation if  $R$  and  $H$  are unlinked ( $RH$ ) =  $1H : 3G$ , if  $R$  and  $H$  are linked ( $\widehat{RH}$ ) =  $1H : 1G$ .

Reference numbers		Form of mating and factorial constitution of parents (Linkage of $R$ and $H$ not shown)	$F$	
Exp.	Family		Hoary	Glabrous
10	1	( <i>incana</i> × 1954) × 1971 <i>v. alba</i>	64	61
	2	" × 1997	18	15
	3	" × 1972	39	32
	4	" × 11001	5	7
	5	" × "	40	40
	6	" × "	56	53
	7	" × 1971	10	11

Of the 219 glabrous plants 99 were flowered. None was sap-coloured.

11	†1	(1887 × 1338) × 1971	(no-d. white × sulph. $A$ ) × sulph. $B$ ( $CK$ × $RHK$ ) × $CK$	6	8
	3			29	20
	4			14	10
				20	17

Of the 55 glabrous plants 33 were flowered. None was sap-coloured.

12	1	(1327 × 1677) × 1991	(sulph. $A$ × d.-white) × sulph. $B$ ( $RHK$ × $CK$ ) × $CK$	37	41
	2	" × "		55	65
	3	" × 1971		16	13
	4	" × "		65	57

Of the 176 glabrous plants 120 were flowered. None was sap-coloured.

13	†*1	(1342 × 31156) × 3879	(sulph. $B$ × ext. no-d. cream) × sulph. $B$ ( $CK$ × ext. $RHK$ ) × $CK$	8	23
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Of the 23 glabrous plants 17 were flowered. None was sap-coloured.

14	1	1145 × (1338 × 1954)	ext. no-d. white × (sulph. $A$ × sulph. $B$ )	40	36
	†2	1146 × "	ext. $C$ × ( $RHK$ × $CK$ )	14	23
	†3	1147 × "		9	20

Of the 79 glabrous plants 51 were flowered. None was sap-coloured.

Expectation of  $R$  and  $H$  are unlinked ( $RH$ ) =  $1H : 7G$ , if  $R$  and  $H$  are linked ( $\widehat{RH}$ ) =  $1H : 3G$ .

15	1	1144 × (1554 × 1954)	ext. no-d. white × (no-d. red × sulph. $B$ )	6	16
	2	1146 × "	ext. $C$ × ( $GRH$ × $CK$ )	13	43
	3	1147 × "		29	75
	4	1148 × "		12	53

Of the 187 glabrous plants: 30 were not recorded  
63 were sap-coloured  
94 were non-sap-coloured

16	*1	1159 × (41287 × 40153)	ext. no-d. white × (ext. no-d. white × no-d. red) ext. $C$ × (ext. $K$ × $CRH$ )		
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Of the 28 glabrous plants: 9 were sap-coloured  
19 were non-sap-coloured

TABLE II (*continued*).

Reference numbers			Formation of mating and factorial constitution of parents	F'	
Exp.	Family	Parents	(Linkage of <i>R</i> and <i>H</i> not shown)	Hoary	Glabrous
17	1	11411 × (41102 × 4131)	ext. no-d. white × (ext. no-d. white × ext. no-d. white)	4	17
	*2	1143 ×	ext. <i>C</i> × (ext. <i>RHK</i> × ext. <i>C</i> )	1	28
	*3	1144 ×		8	50
	4	1145 ×		32	71
	5	1147 ×		8	22
	6	1148 ×		11	25
	*7	1149 ×		3	20
			Of the 233 glabrous plants: 73 were not recorded 58 were sap-coloured 102 were non-sap-coloured		
18	*1	(41102 × 4131) × 1143	(ext. no-d. white × ext. no-d. white) × ext. no-d. white	2	10
	2		(ext. <i>RHK</i> × ext. <i>C</i> ) × ext. <i>C</i>	13	45
	3			6	14
	4			19	37
	5			8	21
	6			17	66
			Of the 193 glabrous plants: 75 were not recorded 45 were sap-coloured 73 were non-sap-coloured		

Expectation if *R* and *H* are unlinked ( $RH$ ) =  $9H : 7G$ , if *R* and *H* are linked ( $\widehat{RH}$ ) =  $3H : 1$

19	1	(1554 × 1954) × (1338 × 1954)	(no-d. red × sulph. <i>B</i> ) × (sulph. <i>A</i> × sulph. <i>B</i> ) ( $CRH \times CK$ ) × ( $RHK \times CK$ )	11	:
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Both glabrous plants were non-sap-coloured.

also contain *H*, it must be said that proof that a *CHK* white strain was actually employed or that it exists has not yet been fully established.

Although linkage between *R* and *H* has been found to be of very general occurrence, it is apparently not universal, as shown by some of the results published in 1916, when two cross-breds obtained by mating two extracted ( $F_2$ ) individuals containing *CRH* and *K* respectively yielded the proportion of hoary and smooth which we expect when the parents are heterozygous for four *unlinked* factors, the anticipation in this case being  $81H : 175G$ , the numbers actually recorded in the two families being  $17H : 33G$  and  $67H$  and  $135G$  respectively<sup>1</sup>. In another case where an  $F_1$  cross-bred from the mating *CK* sulphur-white × extracted<sup>2</sup> *RKH* cream had been crossed back with a *CK* sulphur-white, the  $F_2$  generation consisting of  $8H$  and  $23G$  agreed with the expectation  $1H : 3G$  which is realised in such matings when none of the factors are linked. In a third experiment, where two sister *CK* sulphur-whites had been crossed with *RHK* pure-bred cream, and where, therefore, in the

<sup>1</sup> *Journ. Genetics*, loc. cit. p. 150.

<sup>2</sup> From a mating between glabrous no-d. cream and glabrous d. light purple.

absence of linkage we should expect a ratio of  $27H : 37G$ , eight of the resulting  $F_1$  cross-breds gave ratios approximating to  $9H : 7G$ , but the remaining cross-bred yielded  $43H$  and  $54G$ , or  $26.9H : 37G$  (see Table I, Exp. 1). Presumably in most of the pollen grains of the male parent  $RH$  was linked ( $\widehat{RH}$ ), but in some few unlinked ( $RH$ ), just as the gametes of a non-double-throwing cross-bred derived from an eversporting ( $XY$ )  $\times$  a pure-breeding ( $\widehat{XY}$ ) single have been supposed to be of two kinds— $\widehat{XY}$  and  $XY^1$ . The further fact that  $F_1$  from white *incana*  $\times$  *CK* sulphur-white crossed back with the male parent gave approximately equal numbers of coloured hoary and glabrous whites and creams ( $232H$ ,  $219G$ )<sup>2</sup> shows that linkage also occurs between  $R$  and  $H$  when the whole factor group  $CRHK$  is present (as it is in all hoary types), as well as in the 3-factor glabrous strains  $CRH$  and  $RHK$ .

As data indicating linkage in certain pure-bred strains between  $R$  and  $H$  gradually accumulated, it became desirable in the light of the new facts to re-examine some of the earlier results which had been adduced in support of the occurrence of the expected ratio. In the account given in the *Journal of Genetics*, 1916, p. 149, seven families of various parentage are cited as exhibiting the ratio, in accordance with expectation, of  $27H : 37G$ . With regard to  $F_1$ , a correction has now to be made in respect of the parentage, which by an oversight is there stated to be  $CRH \times RK$ , but which was in fact  $CRH \times CK$  (both individuals, it may be remarked, being  $F_2$  derivatives of a cross), a replacement which, however, does not affect the ratio of hoary to smooth, which will presumably be the same for both unions<sup>3</sup>. In  $F_2$  the genealogy was also complex. Neither parent was pure-bred, and one at least was heterozygous. From evidence obtained later it seems not out of the question that the gametic union was in reality  $RHK$  (extracted)  $\times$   $CK$  and not, as supposed  $HK \times CRK$ . Such replacement again would not necessarily involve an altered expectation, but it precludes the assumption that  $HK$  (without  $C$  or  $R$ ) had been obtained from the operation in question. In the remaining five families only pure-bred individuals were employed, with the exception of an extracted cream known to contain  $R$ ,  $H$  and  $K$ , and these families alone<sup>4</sup> have been included in the tables accompanying

<sup>1</sup> *Journ. Genetics*, Vol. I. No. 4, p. 335, 1911.

<sup>2</sup> See Table II, Exp. 10.

<sup>3</sup> A similar substitution of  $C$  for  $R$  is entailed in the formula for the parentage of the glabrous whites employed in Exps. 8, 16 and 18 of Table I, *Jour. Genetics*, Vol. x. pp. 175-177, 1920.

<sup>4</sup> One of these is also omitted as a later sowing brought up the total for this family from  $26H$ ,  $30G$  to  $57H$ ,  $59G$ , a result which left the true ratio still in doubt.

the present fuller account, in which the origin (whether pure-bred or cross-bred) of the parents is recorded in all cases. For it is to be noted that of the 11 families (marked with an asterisk) in Tables I and II, derived from an  $F_1$  in which  $R$  and  $H$  segregated independently, eight certainly (see Exps. 4, 9, 13, 16, 17) and possibly ten (see also Exp. 3) resulted from unions in which *extracted* individuals were employed, thus leaving only a single family among those obtained from parents of known or supposed *pure* descent as evidence of the dissociation of  $R$  and  $H$  in gametogenesis in  $F_1$  from such matings. That is to say, only a single gamete in the case of strains believed to be pure-bred gave rise to an  $F_1$  in which  $R$  and  $H$  segregated independently, this gamete being produced by a plant (1383) of commercial origin whose previous history was unknown. Two descendants by self-fertilisation of this particular individual were, however, used to fertilise the offspring of two inter-fertilised  $CK$  sulphur-whites, and 20  $F_1$  cross-breeds were obtained, but in none of the  $F_2$  families did the numbers of hoary and smooth indicate independent segregation of  $R$  and  $H$  in the  $F_1$  gametes (see Exp. 2). As a pure-bred culture from the original plant (1383) has been maintained up to the present time, it should be possible by repeating the cross to discover whether linkage in the  $F_1$  gametogenesis is complete or partial.

We may now turn our attention to sap colour. In cases where, on account of the small numbers observed, the statistical record must be held indecisive as between a simple factorial or a linkage ratio, it had hitherto appeared possible that in certain unions the desired proof would be furnished by the presence or absence of sap-coloured individuals among the glabrous plants in  $F_2$ . For example, in the mating  $CRH \times CK$  (Table I, Exp. 6) the proportion of hoary to smooth in  $F_2$ , viz. 143 $H$  and 123 $G$ , is in itself sufficient proof that here  $R$  and  $H$  must be linked, since we do not get the full theoretical excess (calculated on the factorial difference) of glabrous over hoary, viz. 27 $H$  : 37 $G$ , but instead numbers approximating to the ratio 9 $H$  : 7 $G$ . That is to say, the  $F_1$  cross-bred, although heterozygous in three factors, behaves as though it were so only in two.

Now of these glabrous plants the expectation is that there will be three coloured, with a few scattered hairs on the stigma and the single terminal hair on the hydathode characteristic of the leaf of  $CRH$  plants, to every four uncoloured and without these hairs. In fact, however, only 42 were coloured (all with the terminal and stigmatic hairs), while 81 were uncoloured, where the expectation is 53 and 70 respectively. Further examination of these 81 whites showed that 15 of them (or

## 112 *Further Studies on Inheritance in Matthiola incana*

about one-quarter) bore one or two stigmatic hairs. These same individuals, when crossed with a form known to contain *K*, yielded all hoary offspring. They must therefore have been *CRH* plants in which the sap colour was suppressed, and when subtracted from the total of whites and creams and added to the actually coloured give us 57 proved to contain *CRH* and 66 lacking the colour couple, or a close approximation to the expected ratio of 3 : 4. It is thus clear that it is possible for *CRH* to be present in a glabrous strain in which nevertheless the flowers are white or cream. With suppression of sap colour in the hoary type we have always been familiar, witness the white form of *incana* which contains all the four factors *CRHK*, but it is now apparent that the manifestation of sap colour may similarly be inhibited in a *glabrous* strain. We must therefore suppose that white *incana* either lacks or (since the flower may tinge on fading) is deficient in some constituent (indicated by *A*) present in the purple type, without which the *CR* couple is not fully effective; and that in one strain of commercial *CK* sulphur-whites employed there is similarly lacking some component (distinct, however, from *A* since when crossed with white *incana* it gives a purple  $F_1$ ) indicated by  $A_1$ , which is to be found in the *RHK* sulphur-whites and creams. We thus arrive at the necessity for postulating the existence of two additional factors which are required for, or which affect colour production. If this be so, then it would naturally follow that one-quarter of the  $F_2$  glabrous plants containing *CRH* in the mating described above, that is to say, all those homozygous for absence of *A*, would be white instead of coloured, as was found to be the case. Furthermore we should then expect one-quarter of the  $F_2$  hoary plants from such a mating also to be non-sap-coloured, and this, too, was observed, the full analysis of the family being as follows:

$F_2$ from ( $\widehat{CRH} \times CK$ ) $\times$ self =			
Hoary		Glabrous	
Sap-coloured	105	Sap-coloured with stigmatic hairs	40
Non-sap-coloured	27	Non-sap-coloured " "	16
Not flowered	9	Non-sap-coloured without hairs	57
		Not flowered	10
Total	141	Total	123

The appearance of *non-sap-coloured*  $F_2$  hoary plants was indeed always noticed when this particular *CK* sulphur-white strain was used in matings with the *CRH* and *RHK* strains.

What has been stated above in regard to the inhibition of colour in certain cases in individuals containing *CRHK* and *CRH* holds also in

similar circumstances for individuals containing  $CRK$ . Thus in Exp. 1 (Table I) where, out of 9  $F_1$  cross-breds from the mating  $CK$  sulphur-white  $\times RHK$  cream, eight yielded approximately  $9H : 7G$  in  $F_2$ , indicating linkage of  $R$  and  $H$ , and where, therefore, naturally all glabrous plants would be white or cream, the remaining cross-bred gave  $43H$  and  $54G$  (family 7), a result approximating to the  $27H : 37G$  which we should expect from such a mating when  $R$  and  $H$  are not linked. But in this latter case we also should expect among the glabrous plants a ratio of 9 containing  $CRK$ , and therefore coloured, to 28 uncoloured, whereas in fact all the 42 which were flowered were colourless. If the ratio of  $27H : 37G$  in this case is genuine, and the evidence is strongly in favour of its being so, for it so happened that counts were made at intervals as germination proceeded and the successive records  $32H$   $39G$ ,  $41H$   $53G$ ,  $43H$   $54G$  are uniformly consistent, it still remains to be explained why an  $RHK$  strain with  $R$  and  $H$  unlinked gives no coloured  $CRK$  in  $F_2$  when crossed with a  $CK$  strain.

#### SUMMARY OF CONCLUSIONS.

To the conclusions already recapitulated above (pp. 101 and 102) we can now add the following:

1. One of the two factors for sap colour (taken to be  $R$ ) is linked with one of the two factors for hoariness (taken to be  $H$ ) in all the pure-bred commercial strains containing  $R$  and  $H$  which have so far been employed viz. white *incana* ( $\widehat{CRHK}$ ), and glabrous strains both coloured ( $\widehat{CRH}$ ) and uncoloured ( $\widehat{RHK}$ ).

2. Hence when these strains are crossed with a  $C$ -containing form, the  $F_2$  generation contains a smaller proportion of glabrous individuals than the actual factorial difference would lead us to expect. Thus from an  $F_1$  heterozygous for all the four factors,  $C$ ,  $R$ ,  $H$  and  $K$ , where the expectation is  $81H : 175G$ , we get the lower ratio  $27H : 37G$ . Where a 3-factor difference leads us to anticipate  $27H : 37G$  we get  $9H : 7G$ ; and the  $F_1$  heterozygous for two factors yields  $3H : 1G$  in place of  $9H : 7G$ . From a back-cross in like manner we get  $1H : 3G$  where the factorial difference leads us to expect  $1H : 7G$ , and  $1H : 1G$  in place of  $1H : 3G$ .

3. On the other hand, some of the gametes of derivatives from matings in which  $\widehat{CRH}$  and  $\widehat{RHK}$  strains are used, apparently carry  $H$  without  $R$  and  $R$  without  $H$ , since some  $F_2$  families thus bred exhibit the ratio of hoary and smooth which we expect when no linkage occurs.

4. In addition to *C* and *R*, some further component (indicated by *A*) is required to produce sap colour, which is lacking in white *incana*, a form which contains *CRHK* but is nevertheless white-flowered though the petals sometimes tinge on fading. That *A* must be considered to be an essential component which is lacking rather than an inhibitor which is present, is deduced from the fact that *incana* type  $\times$  *var. alba* gives purple  $F_1$ .

5. It must furthermore be supposed that the manifestation of sap colour is dependent upon yet another factor (indicated by  $A_1$ ) which is lacking in some commercial *CK* sulphur-whites. Hence when such a strain is used in a cross with forms containing *RH*, as e.g.  $\widehat{RHK}$  and  $\widehat{CRH}$  glabrous strains, a proportion of the  $F_2$  hoary and *CRH* glabrous are white flowered. That  $A_1$  is not identical with *A* follows from the fact that white *incana*  $\times$  such *CK* sulphur-white gives purple  $F_1$ .

6. The precise part played in colour production by *A* and  $A_1$ , and their relation to *C*, *R*, *H*, and *K* is not yet wholly clear, for although no difficulty is involved in the case where  $\widehat{CRH} \times CK$  gave an  $F_2$  with *one-quarter* of the *CRH* plants white-flowered, some further explanation is required to account for the family where *all* the  $F_2$  glabrous plants were uncoloured, although the constitution of the individual mated with *CK* appeared not to be  $\widehat{RHK}$ , from which we should have expected this result, but *RHK*.

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# VARIATION AND HYBRIDIZATION IN *ISOKONTAE* AND *AKONTAE* IN RELATION TO CLASSIFICATION.

BY W. B. CROW, M.Sc., PH.D. (Lond.),

*Lecturer in Botany, University College of  
South Wales and Monmouthshire.*

ALTHOUGH the natural classification of a group of animals or plants must ultimately rest on a review of all their characters, the latter are not all of equal value in systematic biology. Amongst the Algae the characters used for distinguishing the larger groups, the classes, the following are generally regarded as important: the nature of the reproductive cells, especially their symmetry, and, if motile, the characters of their flagella; the microchemical and morphological nature of the cell wall; the characters of the chromatophores, especially their pigmentation; the nature of the metabolic products of the cell, especially of the chief assimilation product. It also seems that the intimate characters of the nucleus might be used for distinguishing these larger groups, since H. von Neuenstein (11) has shown that its main variations in essential internal structure are largely parallel with the other important group distinctions, but in actual systematic practice the nuclear characters are not readily determinable. It will be noted that all the characters mentioned above are those of the individual cells.

The subdivisions of the classes, the orders, are on the whole distinguished among themselves by very different characters from the above. Here we shall deal only with the divisions of the two classes or subclasses *Isokontae* and *Akontae*. Without going into the details of the various systems that have been proposed, it will be possible to cite certain characters which are very generally regarded as to a large extent determinative of the orders. Such are the degree of colony formation and its nature; the degree of development of the coenocytic habit; the relative duration of the different phases in the life history; the degree of sexual differentiation, if any; the degree of division in the sporangium or gametangium. These characters, together with certain minor features of the cell, are also used in the determination of the families, the latter of course showing somewhat less variation in them. Finally among



generic and specific differences are numerous changes in detail of form in chromatophore, cell, colony and thallus.

It is clear that any discussion of the relative stability of such characters during the course of phylogenesis must take into account the variations and recombinations which they have actually been observed to undergo. It is only then that a critical estimate of the relative permanence of the systematic characters may be made. The following, whilst not intended to be a complete compilation of the relevant data, is an account of some of the phenomena which have a direct bearing on the subject.

It would seem that in Green Algae, as in the group of higher plants, both cross and self fertilization frequently occur. In the genus *Spirogyra*, for instance, conjugation does not always take place between cells of different filaments but, as is well known, not infrequently occurs between cells of the same filament. This difference seems to depend partly on inherited factors, since some species of *Spirogyra* tend to show self fertilization of the filaments less frequently than the others. Further, according to G. S. West (22), the members of the closely related genus *Zygnema* show this method of conjugation still less frequently. Self conjugation is here regarded as abnormal, and the attempt may fail. The presence, in cases of self fertilization, of the conjugation tube, which could hardly have been evolved except in relation to cross fertilization, suggests that self fertilization is a secondary condition in the phylogenesis of *Spirogyra*.

That hybridization between different species may have occurred in cross fertilized Green Algae is indicated by several observations. Before describing them it must be pointed out that crossing does not always occur under conditions that would seem to favour it. Thus Archer (quoted by West, 21) referring to Desmids of the genus *Microsterias* stated that "the conjugating specimens always conjugate like form or species with like form or species" even when individuals of the conjugating form are rare and mixed with much more abundant individuals of other species. Archer used this as a strong argument for the validity of the species. Subsequently this observer saw a single zygospore produced by hybridization between two species of the allied genus *Euastrum* (see 22). The development of the offspring was not followed in this case, which is apparently a very exceptional one among Desmids. In fact, according to W. and G. S. West (25), "there is no doubt that conjugation frequently takes place between two individuals which have just separated by vegetative division, the two new semi-cells being as yet only im-

perfectly developed. This is frequently noticed in large species of the genus *Closterium*, such as *Cl. moniliferum* and *Cl. Ehrenbergii*, also in *Microsterias denticulata* and in species of *Cosmarium* and *Euastrum*." In the filamentous desmid genus *Spondylosium* G. S. West (22) records fertilization of one cell by another adjacent to it in the same filament. Of course, even in these species, the possibility of occasional crosses is not excluded. Transeau (19) also points out that hybridization is very rare in the *Zygnemaceae*; of 854 North American records of *Spirogyra* made by this investigator only five showed hybrids.

The Akontae are particularly favourable for observations on hybridization because of their mode of sexual union. Bessey in 1884 (3) first called attention to the process in *Spirogyra* where he observed a cross between *S. majuscula* and *S. protecta*. The resulting zygospore was of the same spore type as the female parent. This is always the case in *Spirogyra*. As Transeau (19) remarks, this cross is interesting because *S. majuscula* has plain end walls and *S. protecta* has replicate ones, so that the two species would fall into two distinct systematic subdivisions of the genus. B. Cunningham (5) however recently noted the occurrence of both types of end wall in a single filament.

In 1898 W. and G. S. West (24) recorded a cross between two small unidentified species, and in 1911 Andrews (see 4) one between *S. crassa*, which has very large cells with several chromatophores, and *S. communis* which has very small cells with one chromatophore. Unfortunately the characters of the offspring were not observed in these cases. Transeau (19) cites *S. maxima* var. *inaequalis* as a hybrid between *S. maxima* and probably *S. nitida*. Pascher (14) also observed hybridization between two species of *Spirogyra*.

The occurrence of interspecific fertilization in *Spirogyra*, although it is certainly a rare phenomenon, warrants the question as to what extent new types can arise by this method. Transeau (19) has explained the characters of certain types of *Spirogyra* in this way. He found for instance *S. varians* crossing with *S. communis*, and, in the same habitat, forms which combined the characters of these species. *S. varians* differs chiefly from *S. communis* in having much larger cells which become inflated in the conjugating phase. On the basis of observations that in *Spirogyra* the fusion nucleus of the zygote undergoes a mitotic division (20) it is generally assumed that the nuclei of the filaments are haploid. We should then expect, if the two differences between *S. varians* and *S. communis* mentioned above behave as two pairs of allelomorphs, that the immediate offspring of the cross would be of four different kinds, only

two of which would resemble the respective parent forms in both characters, the other two types showing the other possible combinations of these characters, viz. larger cells with no inflations and smaller cells with inflations. Transeau actually observed both these types along with the normal specimens of *S. varians* and *S. communis*. Similar observations were made by Transeau on *S. varians* and *S. porticalis*. Here three specific differences were taken into account (dimensions of cell, form of fertile cell and shape of spore), and it was found that, accompanying the typical *varians* and *porticalis* forms, were fruiting specimens which possessed various combinations of the characters of these two species.

It must be mentioned here that, according to Cunningham (4), meiosis in *Spirogyra* does not invariably take place on the germination of the zygote. He thinks that it generally occurs at this stage because the filaments usually behave as if they were unisexual. But in those filaments which are self fertilized the filaments are at first bisexual, and reduction, with its accompanying segregation of the sex factors, does not occur until the formation of gametes. Cunningham thus explains alternation of male cells (empty after fertilization) with female cells (containing the zygotes after fertilization) in such a filament. He also thinks that reduction sometimes occurs during various stages of the growth of the filament, and uses this to explain cases of so-called cross conjugation, i.e. those in which cross fertilization occurs, but in which each filament of the conjugating pair contains both male and female cells. If sex factors should segregate at various stages in the growth of the filament it is to be expected that factors for other characters would do so. We have already referred to the forms reported by Cunningham (5) in which characters generally believed to indicate different systematic divisions have been found in one and the same filament. These explanations of course depend on the assumption that sex factors in *Spirogyra* behave in a simple Mendelian manner.

Some sixty species of *Spirogyra* are now known. Characters of the same nature as those studied by Transeau and Cunningham are important in distinguishing many of these species, and occur in all sorts of combinations. The allied genera of *Zygnemaceae* are less rich in species, but differ among themselves in much the same way. Thus, size of cell and inflation of the conjugating cells are important systematic characters in *Zygnema*. The possibility of hybridization in the Desmids throws new light on the great wealth of closely related forms found in this group, and may even account for the curious variants occasionally met with in which two different types of chromatophore are found within the

same cell (*Staurostrum*, axile and parietal or semi-parietal types of chromatophore, see 25). The great rarity of hybridization and consequent persistence of type indicate that it will be advisable to continue the use of the term species for the varied forms of Desmids which we now designate as such, even if it should prove that many of them originate by crossing.

The formation of hybrids has been observed in a few members of the *Isokontae*. Pascher (14) records the production of heterozygotes between different species of *Ulothrix* and between a species of *Stigeoclonium* and a species of *Draparnaldia*. He also succeeded in experimentally crossing two species of *Chlamydomonas*. These were not identified with any previously described species but the author simply speaks of them as *Chlamydomonas* I and II. The former was easily induced to form gametes, and these were mixed with gametes of a large number of other species of the genus as each of the latter became available in the sexual stage. Only with *Chlamydomonas* II however were heterozygotes produced; over 120 attempts with other species being unsuccessful. In the mixture of gametes of *Chlamydomonas* I and II only 3 per cent. of the fusing pairs produced heterozygotes. But even this small proportion is remarkable in view of the fact that the gametes of *C. II* possess a membrane<sup>1</sup> whilst those of *C. I* do not. The heterozygotes were intermediate in structure between the two homozygotes. Cultures were made from the heterozygotes. These cultures were found to be of two kinds which may be spoken of as (a) and (b).

In cultures of the (a) kind only organisms of the two parent types were to be found. These two types occurred in varying proportions which Pascher explained by the fact that they multiply at different rates. Direct observation of the germinating zygote showed that of the four cells proceeding from the zygote there are two of each parent type. It is remarkable that both chromatophore forms were present in the offspring, as most writers assume that one chromatophore disappears in the zygote. This assumption may not hold for *Chlamydomonas* being based on *Spirogyra* where only one of the four products of the reduction division develops. In the *Chlamydomonas* culture (a) there is no proof that the nuclei fused in the zygotes. Nuclear fusion was observed in thirty-five cases, but in all of these, of course, it was necessary to kill and stain the cell.

In cultures of type (b) Pascher found organisms of the parental types,

<sup>1</sup> The enveloped character of the gametes is a feature of several species of *Chlamydomonas*.

although slightly modified, and also two intermediate types. The germination of some heterozygotes also showed offspring with new combinations, but such heterozygotes did not reproduce the parent form. Certain of these combinations did not occur in (*b*) cultures, and have not been cultivated successfully. The following tabular view of the characters of the parent forms and the offspring as occurring in (*b*) cultures is given by Pascher:

	Form	Membrane	Papilla	Chromatophore	Eye-spot
Parent I	Pear-shaped	Delicate	None	Lateral	Linear
Parent II	Spherical	Coarse	Present	Basal	Broad
Offspring i	Pear-shaped	Delicate	None	Lateral	Linear
Offspring ii	Pear-shaped	Delicate	None	Basal	Broad
Offspring iii	Ellipsoidal	Coarse	Present	Lateral	Linear
Offspring iv	Spherical	Coarse	Present	Basal	Broad

If reduction had in this case taken place on the division of the zygote, as it is supposed generally to do in *Chlamydomonas*, then the distribution of the characters is quite that which would be expected if segregation takes place in the usual way. Pascher states that the chromosomes, not more than ten, were present in equal number in these new forms as in the parents. The chromosomes are all alike so that it is not possible to say how they are distributed in the new forms. The latter illustrate the possible origin of new species, constant at least as far as vegetative division is concerned, and this is the chief mode of reproduction for haploid organisms.

Pascher points out that there is a great difference between hybrids resulting from crossing two diploid organisms, and hybrids of the present type. The latter resemble those gametes produced from a heterozygote which possess new combinations of characters not found in the gametes of either parent. They are only formed as the result of interaction of more than one pair of allelomorphs. Hybrids of the ordinary diploid type are designated amphimiktic by Pascher, the process being termed amphimixis. The author calls the new combinations obtained in predominantly haploid organisms, such as *Chlamydomonas*, haplomiktic, and speaks of the process as haplomixis.

The characters which differentiate the forms of *Chlamydomonas* in the above-mentioned experiments are of the same nature as those which constitute specific distinctions in the genus. In fact, the form of cell, thickness of membrane, form and position of chromatophore and eye-spot are among the most important taxonomic characters among the *Chlamydomonadaceae*. In view of the observations cited above it seems possible that many of the forms of both *Isokontae* and *Akontae* described by systematists may have arisen suddenly by hybridization. Other re-

arrangements of the hereditary material are not excluded, but we yet know very little of such variants in Green Algae. In several *Akontae* (24, 25) it has been found that occasionally three or even four cells fuse together successfully to form a zygospore, but the germination of these abnormal forms is unknown.

Apart from the new forms which may arise by hybridization and concerning which, it must be emphasized, so little is known at the present time, there are numerous variants among *Chlorophyceae* which seem to owe their origin to other phenomena. Where there appears to be a relatively clear distinction between individual and specific variation, the nature of the individual variant types can be compared and contrasted with the specific types. We will omit numerous observations where no obvious distinction can be drawn between these two classes of variation. The very extensive researches which have been carried out during recent years with pure cultures of the simpler *Chlorophyceae* have shown that very minute morphological and physiological differences may be transmitted for many thousands of generations without change. Thus G. M. Smith (18) working on *Scenedesmus* found that numerous species distinguished by systematists, and even forms which had been regarded as at most of varietal rank, remained constant in culture if conditions were stable. Numerous experiments also prove that in many cases a change in the nature of the medium, or in other environmental factors, produces a corresponding morphological or physiological change in the individuals. It is unnecessary to dwell on these facts, which agree exactly with what has been established for other groups, and do not help to explain the origin of specific differences.

Every physico-chemical change taking place in the organism tends to produce a permanent modification in its protoplasm. This is undoubtedly one aspect of evolutionary change. Even hybridization involves the combination of germ-plasms of different compositions, and is therefore likely to have physico-chemical effects which can be considered apart from the mere fact of combination. Since in the *Chlorophyceae* a highly developed soma never occurs, the part played by such changes is likely to be more obvious than it is in the higher organisms. But the origin of any permanent change other than that produced by crossing as described above, has not been observed in cultures of the Green Algae, and would be very difficult to prove. It seems nevertheless that the problem may be approached by examining the nature and mechanism of the relatively transitory change, and comparing it with the nature and mechanism of the specific differences. The following is an attempt to see how far this is possible.

The study of variation in the unicellular Algae is often rendered difficult by the small size of the organisms and by the fact that even in the more complicated types such as many Desmids the range of variation is comparatively slight. The zoospores of certain *Ulotrichales* however afford suitable material for the study of variation and have been investigated by Pascher (13). In this group individual variants show sufficient difference among themselves to have led authors to distinguish between macro-, micro- and gameto-zoospores, although variants of transitional types also occur. The origin of variation does not appear to lie in the condition of the vegetative form since, according to Pascher, the abnormal forms of *Stigeoclonium* and *Ulothrix*, which at times arise under peculiar conditions of life, produce swarmers like those developed by normal representatives of these genera. The size of the sporangia also does not appear to be a factor influencing variation of the swarmers. Iwanoff in 1899 (see 13) suggested that cells at different stages of development give rise to different types of zoospore. But observation does not confirm this (13). Size of sporangial cell apparently only affects the number of swarmers and not their size. Thus in *Stigeoclonium fasciculare* (13) cells of side-branches produce single macrozoospores, whilst cells of the main axis give two macrozoospores of the same size as those produced from the side branches. The fact that the individual zoospore types cannot be distinguished by size alone shows that cell size is not the cause of this distinction of types. Further investigations on this question however are desirable. Finally the variation does not seem to owe its origin to periodic phenomena. For although in cultures of these Algae macrozoospores appear first, and are followed later by microzoospores, in the intermediate period both kinds are formed, but the intermediate types are not more frequent. Instead, there is a greater frequency of sporangia producing both types during this intermediate period.

Although the characters of the zoospore do not therefore appear to be directly related to their mode of origin, yet their variations are not of course fixed in the next generation. Each zoospore type is capable of reproducing the others through the vegetative plant which it produces on germination. In this connexion it must be noted that the gameto-zoospores are not absolutely dependent on the sexual process for further development, but have been observed to develop independently (13). The differences between the zoospore types thus appear to be of a different nature from the specific differences. And yet the characters which differentiate the zoospore types are very closely comparable with the

characters which distinguish the various species of *Chlamydomonadaceae* and *Polyblepharidaceae*, families which undoubtedly include some of the closest motile unicellular relatives of the *Ulotrichales*. In *Ulothrix zonata* Kuetz, for instance, larger zoospores having an anterior eye-spot can be distinguished from smaller zoospores and gametes having an eye-spot in the middle of the body (13). This variation in position of the eye-spot is also found in motile cells of the genus *Chlamydomonas*, but not amongst individuals of one and the same species of the genus. Hence the character is important here in classification. Another frequent difference between the zoospore types in *Ulotrichales* is in the number of flagella, which is either two or four, many species having both types. It is well known that among the motile *Chlamydomonadales*, the number of flagella is a determinative generic character, the genus *Carteria*, for instance, differing in no essential respect from *Chlamydomonas* except in being provided with four flagella instead of two. Now in *Ulothrix*, according to Janet (10), the quadriflagellate swimmers are diploid and the biflagellate ones haploid. If this is correct it is possible that certain differences in flagellum number among the *Chlamydomonadaceae*, and even the flagellates, may go hand in hand with, or may be of similar nature to differences in chromosome number. This raises the question as to how far the flagellum equipment of the zoospore can be regarded as of fundamental importance in the classification of the Algae. In the majority of recent systems it has been accorded a very high value.

It would appear however that we must distinguish mere doubling of flagellum number and even perhaps other numerical changes from variations of a more fundamental type. On the one hand we have phenomena like the occurrence of 2- and 4-flagellate swimmers in species otherwise of closely similar character. The criticism of Ohno (12) directed against the high systematic value of the flagellum characters in the Flagellata appears to be based on a case of this kind. This investigator discovered a species of *Gymnodinium* which differs from all other members of the genus, and in fact from all other *Peridinales*, in having two longitudinal flagella, although the transverse flagellum was as usual single. On the other hand we have different modes of insertion of the flagellar apparatus, and various kinds of differentiations among the flagella themselves. Thus it is difficult to see how the flagellar apparatus of the zoospore of the *Heterokontae* could be derived directly from that of the *Isokontae*. Even the *Akontae* seem to be completely separated from the *Isokontae* in the characters of their reproductive cells. For although flagella might very readily be suppressed, reversions to the flagellate



forms would probably appear in at least some members of the group if this had occurred in any comparatively recent phase of phylogenesis, just as they do among the reduced immotile members of the *Isokontae*. This however is not the case, and the mode of reproduction in *Akontae* is such that relationship with flagellate forms seems remote. Just as the nuclear apparatus differs qualitatively in the greater groups (11), so the flagellar equipment is very distinct. Simple quantitative differences however may exist in the flagellum number of related species, just as quantitative differences among the chromosomes are well known to exist in closely related forms. The tendency to base the classification of the Algae on the characters of the zoospore is one example of the growing recognition of the importance of cytological characters in taxonomy.

The characters of the multicellular body must not be neglected. In many groups of Chlorophyceae however the species or other sub-groups are distinguished by the characters of the colony. The latter are even more plastic than those of the cell. Thus several colonial forms, such as *Coelastrum* and *Scenedesmus*, have been shown to pass into the unicellular state if grown in appropriate media (15, 22). On the other hand, they remain constant for many generations if the environment is stable. G. M. Smith (18) found that not only minute structural differences in the cells of many species of *Scenedesmus* could remain unchanged, but under these circumstances the number of cells in the colony was also constant within certain limits. Smith also considers the form of colony to be constant, and describes a genus *Tetradesmus* (17) which is closely related to *Scenedesmus*, but in which the four cells, when viewed from the side, are seen to be arranged in two tiers, whereas in the four-celled forms of *Scenedesmus* the cells are all in a single plane. *Tetradesmus wisconsinensis* G. M. Smith is not in other respects distinguishable from *Scenedesmus acutus* Meyen. Yet although Smith grew both these species in parallel cultures under very various conditions for over nine months, the specific colonial characters remained unchanged. In the same way configurations of the colony in *Scenedesmus* are capable of persisting for very numerous generations, and many other instances might be quoted.

The relation of the characters of the colony to those of the cell has been studied by Harper in *Gonium* (7), *Pediastrum* (8) and *Hydrodictyon* (6). In each of these genera development begins with division of the protoplast within the cell wall of each parent cell. In *Gonium* the young cells thus formed remain attached to form the young colony. In *Pediastrum* and *Hydrodictyon* a number of separate swimmers are produced. In the former genus they are extruded in a mucilage bladder in

which, after a period of swarming, they arrange themselves to form the young colony, whilst in the latter the same process of swarming and arrangement takes place within the large parent cells.

In *Hydrodictyon* it was shown that the characters of the colony can be explained solely by the properties of the individual cells. In *Gonium*, on the other hand, because of its mode of reproduction, there is no evidence that the structure of the germ plasm does not represent the space relations of the parts of the adult. It is a close approximation to a pure mosaic development. It is quite unnecessary to assume, however, as Harper points out, that in the cells of *Gonium* there is any complex specific representation of the structure of the colony as a whole. The characters of *Pediastrum* can, according to Harper, be classified into three groups according to the degree of directness of transmission in inheritance. To the first group belong cell characters that are transmitted directly, such as the green chloroplast colour, for example; to the second adult cell characters, somewhat indirectly transmitted, not being visible in the germ cell. The characters of the lobed cell form are of this class. A third class comprises colony characters which have an entirely indirect transmission such as the arrangement of the cells. We have already become familiar with characters of the first and second kind in dealing with unicellular forms. But Harper showed that the characters of the third group could be explained by factors, some of which were due to the characters of the cells, and some to the interaction between the cells. The fact that the cells were aggregated in colonies has an effect on the form of the cells themselves. A given cell form is explained as the response to the position it normally occupies in the colony. Such a form character is however inherited, as is shown from a knowledge of certain abnormal colonies in which cells not in contact with one another may yet develop the characteristic cell form. We thus have in *Pediastrum* an excellent example of the inheritance of a character originally acquired in response to a specific stimulus and now apparently developing without the original stimulus. This appears to be a very general phenomenon in organisms, as has been shown by Semon (16). Whether it has any bearing on the question of the inheritance of acquired characters in the soma of the higher animals and plants cannot be discussed here.

Harper has also analyzed the nature of the specific differences in the genus *Pediastrum* (9). He points out that evolution in the whole genus has proceeded by modification of cell form, and shows how the characters of the members of the various subgenera can be explained as due to contact and pressure responses that have become heritable.

Harper thinks that the subgenera themselves, which are distinguished by the different numbers of spines with which the cells are provided, must either be traced back to a primitive spineless form (of the type of *P. integrum*), or have originated from one another by a sudden change of the nature of a duplication. It may be pointed out however that such a transition as from a one-spined to a two-spined condition might be brought about very easily by a process of incomplete division of the protoplast, which is multinucleate in the mature state in *Pediastrum*, it being only necessary to assume that this takes place before the formation of the firm cell wall.

The mnemonic principle of Semon (16) elucidates many of the systematic differences among the simple Algae. As an example we may take the distinction between the genera *Chlorococcum* and *Chlorella*. This principally consists in the fact that the asexual reproductive cells of the former are motile (zoospores, planospores), whilst those of the latter are immotile (aplanospores). A minor difference is the considerably smaller size of the cells of the latter genus. The cells of *Chlorococcum infusionum* (Schrank) Meneghini are green spheres sometimes attaining a diameter of over  $100\mu$ . Their protoplasts, in the reproductive phase, divide within the cell membrane to form two, four, eight or more zoospores which escape from the membrane, and, after a period of swarming, come to rest, lose their flagella, and surround themselves each with a new membrane. This is the usual method of reproduction, and it occurs when the organism is grown in solutions of certain concentrations (see 2). It can however be modified by altering the conditions of growth. Thus Artari (2) found that in nutritive solutions of particularly high concentration the offspring cells produced by division were immotile, and developed their new cell membranes before escaping from that of the parent. This latter method of reproduction is that characteristic of *Chlorella* where it is of course constant. The difference in size between *Chlorococcum* and *Chlorella* also corresponds with a tendency to vary according to the conditions of the medium, a phenomenon very well illustrated by many unicellular *Isokontae*. The size difference is not however a significant one, since there is a greater difference between the individuals of *Chlorococcum infusionum* amongst themselves than between the average size of individuals of *Chlorococcum* and *Chlorella*. Individuals of *Chlorococcum infusionum* of the maximum size as mentioned above are comparatively rare, the majority ranging as low as  $10\text{--}15\mu$ . In *Chlorella vulgaris* Beyerinck the cell diameter is normally  $5\text{--}10\mu$ . The genera *Chlorococcum* and *Chlorella* are generally placed in separate families.

Other instances might be given of the way in which characters only developed under special conditions in one species appear fixed in others. It would seem that many of the more important systematic distinctions can be looked at from this point of view. A number of the motile *Chlamydomonadaceae*, for instance, are known to pass into a palmella phase under certain conditions of growth, yet there are undoubtedly many allied forms which spend the greater part of their life cycle in a similar palmelloid phase. The experimental investigation of these forms is however still in its infancy.

In conclusion it would appear that the differences between the various species and other systematic subdivisions of the *Isokontae* and *Akontae* are sometimes quite compatible with an origin by hybridization, and in other cases are more nearly analogous with those brought about by special conditions of the environment. We have at present no knowledge of the mechanism of these latter changes, but there is no reason for supposing that it will differ fundamentally from that known in other types of variation. It is probable that the former comparison holds good for many of the finer distinctions, such as those between species and genera; the latter may frequently elucidate the broader divisions such as those between families and orders. As would be expected, few of the variations we have discussed throw any light on the distinctions, apparently closely bound up with the intimate architecture of the protoplasm, which form the foundation of the classes.

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# GENETIC AND CYTOLOGICAL STUDIES IN WHEAT. I.

By A. E. WATKINS, B.A.

*Plant Breeding Institute, Cambridge.*

## CONTENTS.

	PAGE
Introduction . . . . .	129
Material and methods . . . . .	132
Behaviour at reduction from the end of the heterotype prophase to the formation of the microspores . . . . .	133
The fate of the lost univalents present in the microspores . . . . .	142
List of the chromosome counts in the $F_2$ and $F_3$ generations from these crosses . . . . .	143
The frequency of the various gamete classes . . . . .	146
The composition of the population resulting from self-fertilisation of the plants under consideration . . . . .	151
Sterility . . . . .	154
Summary and conclusions . . . . .	155
Appendix . . . . .	158
Tables . . . . .	159
Bibliography . . . . .	165

## INTRODUCTION.

THE seven or eight species into which the genus *Triticum* is now generally divided fall naturally into three groups. The first group contains the single species *T. monococcum*; the second, or "Emmer" group, the four species *T. dicoccum*, *T. durum*, *T. polonicum*, and *T. turgidum*; the third, or *vulgare* group, the species *vulgare*, *compactum*, and *spelta*, to which may be added certain forms previously included as *T. vulgare* and now grouped together under the name *T. sphaerococcum* (Percival).

*T. monococcum* is sharply differentiated from the other wheats morphologically; it is crossed with them only with difficulty; and when hybrids are obtained they are completely sterile. This species will not be considered further.

The species of the second group are fairly clearly defined, but forms more or less intermediate in type can be found, especially between the species *durum* and *turgidum*; all four species cross readily with one

another, and segregation always seems to take place in simple Mendelian fashion. In most cases the hybrids are completely fertile, but  $F_1$  plants from a cross made in 1921 between a variety of *T. dicoccum* and a variety of *T. turgidum*, show in some cases a partial failure to set grain; this case will be dealt with more fully in a later paper.

In the third group the separation of *T. compactum* from *T. vulgare* is probably more or less artificial; there is no difficulty in crossing the two species, and segregation in  $F_2$  is normal, while fertility is complete. *T. spelta* is perhaps more distinct from *T. vulgare*, but it crosses readily with it, the hybrids are fertile and no abnormalities appear.

In any cross, however, between a member of the second group and one of the third the results are quite different. The  $F_1$  is partially sterile; in the  $F_2$  variation occurs between complete fertility and complete sterility, while an immense number of new forms, of almost every conceivable diversity appear, quite unlike either of the parents or any known cultivated variety of wheat.

The present work arose from an attempt to elucidate the manner of the segregation shown by the  $F_2$ , harvested in 1921, of a cross between Rivet, a variety of *T. turgidum*, and Swedish Iron, a variety of *T. vulgare*. A complete  $F_3$  was raised, but it became apparent that the cross could be adequately understood only after detailed cytological investigation.

The earlier observers, Overton (1893), Körnicke (1896), Dudley (1908), Nakao (1911), Bally (1912), found 8 to be the haploid chromosome number in *T. vulgare*, and some of them reported finding 16 in somatic cells. In complete contradiction to these results Sakamura (1918), working with root tips, found the somatic numbers to be 14 for *monococcum*, 28 for *durum*, *turgidum*, *polonicum* and *diococcum*, 42 for *vulgare*, *compactum* and *spelta*. Later, Kihara (1919, 1921) confirmed these numbers for the somatic cells, and found the corresponding haploid numbers of 7, 14 and 21, in the reduction divisions of the microspore mother cells; he also found 35 to be the somatic number in the  $F_1$  of crosses between species having 28 for somatic number and those having 42, and worked out the chromosome behaviour at reduction in plants of the  $F_1$  and later generations. Of other workers Spillman (1909) states that wheat has 40 or more chromosomes, and Sax (1918) found approximately 28 in the first division of the fertilised egg-cell of Kubanka, a variety of *T. durum*.

In view of the greater completeness of the work of Sakamura and of Kihara, of the confirmation provided by the work of the latter on hybrids between forms belonging to the Emmer and *vulgare* groups respectively,

and specially the correspondence between the numbers found by them and the results obtained when the various species are crossed, there would seem to be little doubt of their correctness. More recently Sax (1922) has published an account of the chromosomes of the various wheat species and of their behaviour when species belonging to different groups are crossed, and his results agree with those of Kihara. In viewing the contradictory results of the earlier observers he affirms that in his opinion the figures illustrating their papers are not convincing and attributes their results to faulty fixation. With this opinion I am in complete agreement in so far as the papers I have been able to examine are concerned—those of Overton, Körnicke, Dudley and Bally. Not only is it evident that the later investigators were unduly influenced by the results of Körnicke, but for other reasons in addition I consider their results quite unreliable. I may state that my own results on somatic counts in varieties of the species *durum* and *turgidum*, and on heterotype counts in varieties of the species *durum*, *polonicum*, *turgidum* and *vulgare* agree with those of Sakamura, Kihara and Sax; and I consider that the somatic counts 14 for *monococcum*, 28 for *dicoccum*, *durum*, *polonicum* and *turgidum*, and 42 for *vulgare*, *compactum* and *spelta*, can be accepted unreservedly for all true breeding forms of the above species, though it should perhaps be stated that Prof. Percival is of the opinion that *T. compactum* has not always 42 chromosomes.

Before describing my own results it will be convenient to give the essential facts elicited by Kihara in his work on hybrids resulting when various species of the Emmer group are crossed with the species of the *vulgare* group. The  $F_1$  from any cross was shown by somatic counts to have 35 chromosomes, 14 from the one parent and 21 from the other. At reduction 14 bivalents were present and 7 unpaired, univalent, chromosomes. This was taken to mean that the 14 chromosomes from the Emmer parent paired with 14 of those of the other, leaving the remaining 7 from the latter unpaired. The component chromosomes of the 14 bivalents segregate in the usual way and behave quite normally throughout both divisions, but the 7 univalents lag behind the others, travel later to the equatorial plate of the heterotype spindle, and split longitudinally; the halves of the split chromosomes separate and travel to the poles, where they arrive after the halves of the bivalents, and are included in the daughter nuclei. In the homotype division the univalents are again late, but here they segregate at random to opposite poles, and one or more may often fail to reach the pole and be left outside the reformed daughter nuclei. It was conjectured that these lost univalents



subsequently degenerate. As a result of random segregation in the homotype, and of possible loss of chromosomes in this division, it is pointed out that the resulting gametes will have any number of chromosomes from 14 to 21, the numbers 16, 17 and 18 being probably most frequent. Kihara then investigated several plants from the  $F_2$  and later generations, where he found such numbers as 14 bivalents plus 3 univalents, a total of 31 chromosomes, or 17 bivalents plus 4 univalents, a total of 38 chromosomes. Here the behaviour at reduction was the same as in the  $F_1$ —except for the difference in number—the bivalents behaving normally and the univalents in the manner described. Pollen formation was not studied later than the end of the homotype division. Only a few stages in the reduction of the megaspore mother cell were observed, and it was supposed that here these divisions follow the same general course as in the microspore mother cells.

In the paper already referred to Sax has carried out similar work, but beyond describing the reduction divisions in hybrids between *T. monococcum* and *T. turgidum* his cytological observations go little further than those of Kihara. His discussion of the significance of these observations is considered later.

In the crosses I have investigated the cytological behaviour is essentially similar to that described by Kihara, but differs in certain details; it seems best, however, to give a detailed description of all the observations, even where they agree with those of Kihara or Sax, not only because they have been worked out in greater detail and additional facts noted, but chiefly because my object has been to make an exact analysis of the case, and it is necessary to be certain of the validity or otherwise of the assumptions upon which the mathematical treatment rests.

The importance of exact treatment can hardly be over-estimated, and although difficulties have been met with, a number of interesting facts have already emerged, and it is hoped that a complete solution of the problem will be found possible.

#### MATERIAL AND METHODS.

Some of the material upon which the work has been done is from plants of the  $F_3$  of the cross Rivet ♀ × Iron ♂ already mentioned (p. 130); the remainder is from the  $F_2$  of a cross Rivet ♀ × a smooth chaffed beardless variety of *T. vulgare* from Mesopotamia.

Carnoy's fixative was used for fixing complete ears at about the reduction stage. Sections were cut, both longitudinally and transversely,

at thicknesses from  $14\mu$  to  $16\mu$ , and stained in iron alum haematoxylin without a counterstain. The general practice was to cut about a dozen longitudinal sections of each ear to ascertain the stage in reduction in the anthers of flowers from the various spikelets, then to cut transverse sections of those flowers in which reduction was at a stage it was desired to study in detail. It is often an advantage to study both longitudinal and transverse sections of the same flower.

Ripe stamens for determining the percentage of sterile pollen grains were fixed in Carnoy's fluid, and after washing in absolute alcohol were preserved in 70 % alcohol until required for use. They were then dissected out in Belling's aceto-carmin, in which sterile grains appear completely or partially empty.

#### BEHAVIOUR AT REDUCTION FROM THE END OF THE HETEROTYPE PROPHASE TO THE FORMATION OF THE MICROSPORES.

A detailed description will first be given of the reduction divisions of a plant<sup>1</sup> with 31 chromosomes, of which 3 are unpaired and the remaining 28 form 14 bivalents. As the object of the paper is to describe the segregation of the chromosomes, the heterotype prophase will not be dealt with.

After diakinesis, and just previous to the equatorial plate stage, the chromosomes become compact, a bipolar spindle develops, and the chromosomes begin to arrange themselves across its centre (Figs. 1 and 2). Some of them, however, often 3 but sometimes less, lag behind the others (Figs. 3 and 4) and may not reach the equatorial plate until the components of the bivalent chromosomes have begun to separate (Figs. 5 and 7). Their subsequent history shows these lagging chromosomes to be unpaired univalents. In rare cases (Fig. 6) one of the latter may be left off the spindle altogether and take no part in the division. An equatorial plate, of the stage of Figs. 3 and 4, when seen in polar view will show the 14 compact bivalent chromosomes in one plane, and the 3 univalents generally above or below, and often in the next section (Fig. 9). The clearest counts are usually to be obtained now, as if all the chromosomes are nearly on one plane it is generally at a somewhat later stage, when the bivalents are beginning to separate into their components and their number is less clear. Fig. 8, however, shows a case in which all 17 chromosomes are quite distinct.

Next, the constituents of the bivalents separate (Figs. 10 and 11)

<sup>1</sup> Hereinafter called plant A.

and travel to opposite poles (Fig. 13). The univalents do not travel with them but are left on the equatorial plate (Figs. 11, 13 and 14) and show a longitudinal split. Sometimes the separation of the bivalents begins while one of the univalent chromosomes has yet failed to reach the equatorial plate (Fig. 12). The apparently "unclean" separation of the bivalents into their components, that is to say cases where the almost separated chromosomes are still joined by a long slender thread, is not universal. Fig. 62, the corresponding stage in another plant, shows a case where this does not occur. The significance of the appearance seen in Figs. 11 and 12 has not so far been investigated in detail, and it cannot be definitely said whether the effect is due to fixation or whether it corresponds to a real occurrence; if the latter be the case it may be of some importance. A similar observation was made by Sax (1922, p. 518).

After the separation of the previously-paired chromosomes the 3 univalents are left on the equatorial plate, as described already, and in this stage it is possible to count the three sets of chromosomes separately if they are seen in polar view. Fig. 15 shows one such count, the three sets of chromosomes from the same cell being found in two adjacent sections. In one section the 14 halves of the bivalents are seen (Fig. 15 *a*) showing quite clearly the longitudinal split in preparation for the ensuing homotype division; in the next section are seen in one plane the 3 univalents, while the other 14 halves of the bivalents show up very clearly upon focussing down. The members of each set of 14 have been numbered to show that they preserve their positions relative to one another after separating from the bivalent condition. In this plant six clear counts, in each of which there was no possibility of doubt, were made, all showing this 14—3—14 segregation. Fig. 16 shows the only exception to this rule found in the plant; one set of 14 chromosomes, all approximately in the same plane, are found in the adjacent sections drawn in Figs. 16 *b* and 16 *c*; above them, in Fig. 16 *b*, two of the univalents, *u.v.*, are shown; in the third section (16 *a*), all in the same plane, are found the other 14 halves of bivalents and in addition one univalent chromosome (*u.v.*). The latter is recognised by the fact that it does not show the longitudinal split so clearly seen in the halves of the bivalents. This segregation into 15—2—14 is explained by the extreme lagging of one of the univalent chromosomes in such a cell as is shown in Fig. 12. Here, although segregation of the paired chromosomes has begun, one of the univalents is still well above the equatorial plate, and it is evident that in such a case 14 chromosomes will travel to the lower pole, only 2 univalents will be left in the centre, and the other 14 chromosomes will

meet the remaining univalent and therefore show a plate with 15 chromosomes. This is exactly the condition represented in Fig. 16. The fate of this one univalent, and certain other irregularities which may occur in the heterotype division, are described later for another plant; the divisions occurring in the plant now being considered were very uniform, and are therefore more suitable for an introductory description. ✓

In the succeeding stages the chromosomes at the poles lengthen out into thick threads; the univalents, which already showed a longitudinal split (Fig. 13), complete their split (Fig. 14), and the halves of each travel to opposite poles (Figs. 17, 18 and 19). All may reach the poles (Fig. 19), or some of them may lag behind so that they are still to be seen when the chromosomes at the pole are becoming vacuolated and losing their distinctness of outline (Fig. 20). Later, all trace of the individual chromosomes at the poles is lost, a cell wall begins to form, and the halves of the univalents that were late in travelling to the poles may be just in time to be included in the reforming daughter nuclei (Fig. 21), or may be left behind entirely (Fig. 22). The completion of the cell wall and the separation of the two daughter cells proceed concurrently with a resolution of the chromosomes into an apparently continuous network, while one or more halves of univalent chromosomes are left outside as densely staining spherical masses (Figs. 23 and 24). The fate of these latter will be dealt with after the general features of the homotype division have been described; at this point it will suffice to mention that they are shown in some of the following figures (32-50) lying outside the homotype spindle. Sax does not report any case of loss of univalents in the heterotype division. In his first paper Kihara mentions such loss as occurring "In abnormen Fällen...bisweilen..." but in his later paper says: "Verspatete Chromosomen gelangen oft nicht an die Pole."

After the nuclei have remained for a short time in the reticulate condition the chromatin begins to condense into a number of variously shaped deeply staining masses, which lie for the most part round the periphery of the nucleus (Fig. 32). As condensation proceeds the nuclear membrane dissolves, the chromosomes again begin to be distinct and (Fig. 33), a bipolar spindle having developed, they pass by degrees to the equatorial plate, where they are seen in the form of comparatively long thick threads (Figs. 36 and 37). In polar view it is usually difficult to make certain counts, but Fig. 38 shows clearly 16 chromosomes—14 descended from the bivalents and 2 from the univalents—with one univalent left outside. The 14 that were descended from the bivalents split longitudinally and the halves pass to the poles (Figs. 39-46), while

those from the univalents, in number 3 or less according to how many were lost at the heterotype division, are left on the equatorial plate; their fate will now be described.

At first (Fig. 41) they are very long thin threads and no longitudinal split is noticeable; later they contract somewhat, stain more deeply (Fig. 40), and begin to travel to the poles intact and apparently at random (Figs. 42, 44, 45, 46 and 47). Very often, however, one or more of them fail to reach the poles (Figs. 48, 50 and 51), but contract still further, stain very deeply, and are left outside the reformed daughter nuclei (Fig. 51) which therefore contain any number of chromosomes from 14 to 17. The formation of a cell wall and the separation of the daughter cells complete the division (Figs. 53, 54, 55), so that the original pollen mother cells have now formed tetrads of four microspores (Fig. 55). In the many cells examined no certain case of the splitting of univalents in the homotype division was observed, and if this ever occurs it must be very rarely. In Figs. 42 and 43 where splitting may seem to be taking place it is probable that this appearance is actually due to a slight constriction at the point of the V of a single long chromosome.

Before describing the formation of the pollen grains from the microspores, it will be convenient to consider at some length the fate of the chromosomes lost at the heterotype division and their appearance, together with those lost in the homotype, in the cells of the tetrad.

At the interkinesis stage (Figs. 24-31) it is possible to count the frequency with which chromosomes were lost, that is the frequency of cells showing 0, 1, 2 and 3, lost chromosomes. In carrying out this operation only those cells were noted in which the nuclei were uncut, and where there was no other disturbing factor to render observation uncertain, and a careful comparison was always made with the neighbouring sections to make certain that no error was introduced through the cutting off of a small portion of the cell, and with it an odd chromosome. The results of the count are given in Table I (a). It is at once evident from the table that there is little or no tendency for all the chromosomes to be lost together, and the numbers do in fact approximate to what we should expect if the loss were a random one (see later, p 146).

These lost chromosomes can be traced through the various stages that follow up to the homotype metaphase (Figs. 32-34). In a few cases they may lose in some measure their capacity to take up the stain and show a less distinct outline; Fig. 36 may show an extreme case of this, the body in the upper corner of the cell staining very little more deeply than the cytoplasm, and being surrounded by a clear space. This indicates

that occasionally<sup>1</sup> the lost chromosomes may have degenerated by this stage. Occasionally, too, where there are two of them in a cell, these may be seen lying very close to one another, and appearances suggest that at times they may fuse completely<sup>1</sup>. However, in by far the greater number of cases they are still present at homotype metaphase as deeply staining spherical masses (Fig. 35), or as thick threads (Fig. 37) having approximately the same staining value as the chromosomes on the equatorial plate. A count of their frequency at this stage is given in Table I (b) and shows with certainty that there has so far been very little change in their number. The divergence between the numbers found here and those of the previous count, made at interkinesis, is hardly more than is expected on the theory of random sampling.

At early anaphase (Fig. 39) they are present as thick threads and it is evident that should these threads lie near a pole of the spindle they will probably be included with the chromosomes that travel to the poles on the spindle of the homotype division, while should they lie more distantly this will not occur (Fig. 46). Fig. 40 shows one that has probably only just escaped. A count of the lost chromosomes made at homotype anaphase, about the stage of Fig. 46, is given in Table I (c) and shows that the frequency of their occurrence is markedly less than that at homotype metaphase; this alone, in view of the short interval that elapses between the two stages and consequent small chance that wholesale degeneration had occurred, would be strong evidence that many of them had been included with the daughter groups formed as a result of the homotype division. This recovery of chromosomes previously lost would appear to depend simply on their position in the cell and therefore, in all probability, occurs at random. An additional count is given in Table I (d) from observations made at a slightly later stage—about that of Figs. 50 and 51; as would be expected this gives appreciably the same result. The average frequency of the two counts approximates to that found on the theory of random regain if the chance of any one chromosome being regained equals 0.75 (see later). A count has also been made of the loss of univalent chromosomes at the homotype division (Table I (f)). This again does not diverge from expectation if the loss were a random one (see later, p. 147).

It is interesting to notice the changes that occur in the structure and shape of the chromosomes lost in the heterotype from the time of their loss to the time of the formation of the microspores. At the end of the

<sup>1</sup> It must be emphasised that prolonged study shows that even if these interpretations are correct, such cases are extremely rare.

heterotype division they contract to densely staining spherical masses (Figs. 22 and 23). As the interkinetic nuclei become reticulate most of the lost chromosomes stain less deeply and tend to become more or less distinctly vacuolated (Figs. 24 and 30), but a few seem to show an incipient longitudinal split (Fig. 28), though this may in reality only be a stage in vacuolation. As the chromatin of the nucleus contracts to form chromosomes again the lost univalents lose their vacuolation and again become densely staining (Fig. 32); this condition persists in the majority of cases (Figs. 33, 34, 35) until the early anaphase of the homotype division, by which time they have lengthened and stain less deeply. Sometimes, however, this latter change may have taken place by the time of the equatorial plate stage (Fig. 37), and in a few cases, an extreme example of which is perhaps shown in Fig. 36, they stain less deeply, lose somewhat their distinctness of outline, and possibly degenerate. At the stage of Figs. 40 and 46 they are always fairly long, stain less deeply—about the same as the chromosomes undergoing division—and appearances suggest that very occasionally they may have split longitudinally, but it cannot be certified that this is not the result of two chromosomes lying together side by side. A chromosome left out at the homotype division begins to contract and to stain deeply at the stage of Fig. 48, and this figure shows an exactly parallel behaviour in a chromosome lost at the heterotype, which will be seen to have begun contraction, and has also in fact stained almost exactly like the other chromosome lost in the homotype division. Later, the chromosomes lost at the heterotype contract once more to their original condition—densely staining spherical masses (Figs. 49 and 50).

The striking feature of these changes is that they correspond, to a fairly marked extent, with the changes taking place at the same time—from interkinesis to homotype telophase—in those univalent chromosomes which are included in the daughter nucleus of the heterotype. It cannot be stated how far these changes are of real significance, but they are sufficiently striking to suggest that this may be the case, and several hypotheses can be put forward to account for their occurrence. We might suppose, for example, that a chromosome, having started at the beginning of the heterotype division to go through a definite cycle of changes, tends to carry through these changes as a result, to some extent, of properties inherent in itself, and does so even if isolated and not functioning in its proper environment—that is to say, apart from the influence of the surrounding chromosomes, the achromatic figure, etc. Or, alternatively, the changes undergone by a chromosome from

diakinesis to homotype telophase may be merely the expression of the changes taking place in the cell as a whole during this period, and may therefore be expected to occur, in a minor degree, in a lost chromosome as well as in those functioning normally.

*Certain Irregularities.*

The account that has been given applies in its essential features to all the plants so far investigated, but certain irregularities and divergences from this behaviour have appeared and will now be discussed.

We have already seen that in a plant with 14 bivalents and 3 univalents a segregation of 15—2—14 instead of 14—3—14 was found on one occasion when counting the chromosomes in polar view at heterotype anaphase: the explanation of this appearance has already been given. The same irregularity was found to occur with greater frequency, and other irregularities also, in a plant with 32 chromosomes—14 bivalents and 4 univalents (Fig. 67); Fig. 59 shows this, normal, 14—4—14 segregation. Counts showing 15—3—14 chromosomes are explicable in the way already given, and a more extreme divergence 16—2—14 (Fig. 60) in a similar manner—by the extreme lagging of 2 univalents (Fig. 61).

In this and similar instances it is possible that the lagging univalent may pass to the pole with the halves of the bivalents without dividing. In this event it is probable that since it failed to divide in the heterotype such a univalent would divide in the homotype, and that the split halves would pass to the poles with the halves of the descendants of the bivalents. If this supposition be correct this behaviour of the univalent—random segregation in the heterotype and longitudinal division in the homotype—would not affect the frequencies of the gamete classes<sup>1</sup>, which will be worked out later. No definite evidence on this point is to hand.

Fig. 63 shows separation into 14—4—15; in Fig. 63 *a* are the 14 halves of bivalents all lying in one plane and all showing clearly the longitudinal split for the next division, and in the next plane of the same section 4 univalents, while Fig. 63 *b* drawn from the neighbouring section shows 14 halves of bivalents again all showing the longitudinal split, and in addition a univalent chromosome in which this split does not appear. This probably occurs as the result of the splitting of a lagging univalent, such a one as shown in Fig. 62. If this should split at about the time the halves of the bivalents pass it on their way to the pole they might be accompanied by one half of the split univalent, giving

<sup>1</sup> For a fuller discussion see Appendix.



15 chromosomes on one plane as in Fig. 63 *b*, while the other half would remain at the centre of the cell with the remaining 3 univalents, as yet unsplit, giving 4 chromosomes in the centre of the cell and 14 halves of bivalents passing to the other pole as in Fig. 63 *a*. A slightly later stage of this is probably shown in side view in Fig. 64, where one univalent, the halves of which are marked "a," has split and separated into two halves of which one has travelled to one pole a little later than the halves of the bivalents, while the other has remained at the centre of the cell with the remaining 3 univalents, which in the figure are seen already split and beginning to separate. There seems little doubt that at a slightly earlier stage this cell would have shown 14—4—15 segregation. This view of the apparent anomaly is again confirmed by the fact that the 4 cells in which this 14—4—15 separation could be clearly counted are all in a somewhat later stage than the 3 cases where the normal 14—4—14 separation was found, so that it is not unlikely that one univalent should already have split.

Fig. 65 shows a cell in which one univalent has split before the others, and it is clear that a polar view count of such a cell would show 14—5—14 chromosomes at successive levels, which was found to be the case in one instance.

Fig. 66 shows that all but one of the univalents have already split and almost or quite reached the poles, while one is splitting quite near one pole. This is probably the result of the lagging of one chromosome, as in Fig. 61 or 62, being so extreme that it never reached the equatorial plate, but split where it was after the remainder had passed to the poles. Actually the state of affairs in this cell is far clearer than it is shown in the figure, which suffers from the disadvantage of having to be represented in one plane. In another plant the same observation was made in one cell at a slightly later stage (Fig. 68); the halves of the univalent have just separated but are included in one daughter cell, and their appearance suggests that they are each beginning to round off in the typical manner of chromosomes lost at this division. Neither of these figures suggest that both halves of a split univalent are ever included in the same daughter nucleus, but even if this should happen it must be so rarely as probably to be of no practical importance: the two figures we have described are the only cases of their kind noted, although in the 9 plants so far studied many thousand cells at this stage must have been passed under review.

In none of these cases do the irregularities affect the essential features of the segregation of the chromosomes, though they add considerably to the difficulty of finding the chromosome number of any plant. I con-

sider it unlikely that further study will discover any important difference in the behaviour of the chromosomes during these stages, but at the same time this is a possibility of which it would be well not to lose sight. The theory of the individuality of the chromosomes seems so well established that to seek to interpret these irregularities in a manner that does not conflict with it is legitimate. The interpretations put forward have only been given after careful examination of a large number of slides, and are both probable and consistent. The conditions under which chromosomes retain their individuality are not however too well established, and it is known that they may be broken up under the influence of X-rays. In the hybrids we are considering, the physiological balance of the individual cell may perhaps be somewhat unstable, and for this reason it might seem possible that strict chromosome individuality is not maintained. It will be well therefore carefully to consider any evidence that this is the case, but inasmuch as the observations given are consistent with the theory of individuality they may be considered to support it.

The plants to which the above description applied all have less than 35 chromosomes. A plant (hereinafter plant B) possessing 38 chromosomes—17 bivalents and 4 univalents—has been studied in detail, and its behaviour differs in one respect, but it cannot yet be stated whether this difference is typical of plants with more than 35 chromosomes, or is of no general significance. The difference refers to the chromosomes lost at the heterotype division. It will be recalled that it was found that these generally remained outside the homotype division figure until late anaphase, when they might be re-included—at any rate no very great change in the frequency of cells having 0, 1, 2 or 3 of them occurred until then. In plant B, however, it seems probable that chromosomes lost at the heterotype may be included by the homotype spindle when it is first formed, and take part in the subsequent division—or rather, in their case, segregation. The evidence for this is that they are much less frequent at homotype metaphase than they were at interkinesis (Tables II (*a*) and II (*c*)). This might be accounted for by their rapid degeneration, but no signs of this were noted; moreover the frequency of their appearance at homotype prophase, just before the nuclear membrane dissolves, is little different from that at interkinesis, and markedly greater than that at homotype metaphase, which occurs only a very short time later. The actual re-inclusion of these chromosomes within the division figure cannot of course be observed, but it is often difficult to tell, in the stages between those represented by Figs. 33 and

34, whether one or more of the chromosomes are in the division figure or not. It is thought that this evidence is sufficient to justify the belief here put forward. We may also expect that re-inclusion takes place at random (see Tables II (*a*) to II (*d*)).

#### THE FATE OF THE LOST UNIVALENTS PRESENT IN THE MICROSPORES.

We have seen that towards the end of the homotype division the lost chromosomes are present in the cells as deeply staining spherical masses. From the stage shown in Fig. 51 to the time when the division is completed and a tetrad formed (Fig. 55), vacuolation of these chromosomes may proceed rapidly, and, as shown in Figs. 53 and 54, can probably occur in those lost at the homotype as well as those lost at the heterotype. Yet it will be noticed that the respective frequencies of (1) vacuolated and (2) densely staining chromosomes in the cells of the tetrads of plant A (Table I (*g*)), are the same as the frequencies of (1) chromosomes lost at the heterotype without being subsequently regained, and (2) chromosomes lost at the homotype (Tables I (*e*) and I (*f*)). This may indicate that chromosomes lost at the heterotype become vacuolated first.

Starting with the cells of the tetrad several counts have been made for the plant B to show the frequency with which lost chromosomes are present during the early stages of the development of pollen grains from these cells. Tables II (*i*) and II (*k*) show about the same total frequency of lost univalents, but comparison of these with Table II (*e*) shows that already, since the early homotype telophase, there has probably been a slight diminution in this total frequency. Table II (*k*) was compiled from counts made in cells of about the stage of Fig. 69; after this stage the cells enlarge rapidly, the wall becomes much thicker and a pore is formed in it, but I have not been able so far to trace the development of the complete pollen grain. For, as enlargement of the microspore proceeds it becomes increasingly badly fixed in the Carnoy's fluid which was used throughout the 1922 season as a fixative. However, the next table, II (*l*), referring to about the stage indicated by Fig. 71, shows a further diminution in the total frequency of lost chromosomes. The diminution has gone further still at the stage of Fig. 73, when Table II (*m*) was compiled, but further than this it has not been possible to proceed for the reasons given. This diminution I believe to be due to their degeneration after becoming vacuolated, as when in this condition they often become difficult to distinguish from the surrounding cytoplasm; nor is there any evidence that they are re-included in the nucleus of the microspore. It will, however, be necessary to study this point in greater

detail, as it cannot yet be definitely stated that in no case are the lost univalents re-included during these stages.

It will be noticed that the fall in total frequency is due to a diminution in the number of vacuolated chromosomes, which have all disappeared at the stage to which Table II (*m*) refers, while the frequency of densely staining chromosomes has remained practically constant throughout Tables II (*k*), II (*l*) and II (*m*). However, the densely staining chromosomes themselves are beginning to swell up and to become vacuolated by the time stage 3 is reached, as shown by Figs. 73-76, but they have none of them yet reached the degree of vacuolation shown by the original vacuolated chromosomes of stage 1.

To sum up, as the cells of the tetrad separate they contain both densely staining and vacuolated odd chromosomes. During the initial stages of pollen development the latter degenerate and the former begin to vacuolate. Later than this the development has not so far been studied.

Examination of ripe pollen grains from plant B showed that 21.6 % were completely or partially empty.

Until further evidence is forthcoming it will be assumed provisionally that the odd chromosomes degenerate during pollen development, and that the abortion of certain pollen grains is not selective with respect to the various gamete classes dealt with in the later calculations. These assumptions probably do not seriously disturb the results, and will have sufficient validity until the work can be put on a more definite footing.

LIST OF THE CHROMOSOME COUNTS IN THE  $F_2$  AND  $F_3$  GENERATIONS  
FROM THESE CROSSES.

(1) <i>Rivet</i> ♀ × <i>Iron</i> ♂ $F_3$ .				
No. of plant	No. of biv.	No. of univ.	No. of biv. + No. of univ.	Total chr. No.
1-2	14	0	14	28
4-2	14	0	14	28
13-2	19	2	21	40
334-1	14	0	14	28
470-1	14	0	14	28
754-1	18	3	21	39
754-2	18	3	21	39
754-3	19	2	21	40
(2) <i>Rivet</i> ♀ × <i>Mespot. vulgare</i> ♂.				
3	17	4	21	38
10	14	0	14	28
13	16 (or 15)	5 (or 6)*	21.	37 (or 36)
15	14	4	18	32
30	14	3	17	31
31	14	3	17	31

\* It cannot yet be stated definitely whether the number of univalents in this plant is 5 or 6. The sum of the number of univalents and the number of bivalents is certainly 21.

It will be noticed that in the 6 cases of plants having more than 35 chromosomes, the sum of the number of bivalents and the number of univalents is 21. This important fact was noticed by Kihara in the 19 similar cases he investigated. Secondly, in the 3 cases of plants having less than 35 chromosomes the number of bivalents is 14 though the number of univalents was 3 or 4; this also was noticed by Kihara in the 2 such plants he worked on.

There is no evident reason why a plant with 38 chromosomes might not form 19 bivalents, or 18 bivalents and 2 univalents, and similarly with other plants, while a plant with 32 chromosomes might form 16 bivalents or 15 bivalents and 2 univalents. The significance of the fact that this is apparently not the case is discussed later. In the meanwhile I wish to point out that in addition, to the cases where counts have been made, there is other evidence that plants with (say) 15 bivalents and 0, 1 or 2 univalents, or 19 bivalents and 0 or 1 univalent, never occur. For if they did then it would almost certainly mean that plants breeding true to a condition of 15 or 19 bivalents (say) and no univalents would be found, so that in view of the frequency with which crossing between 28 and 42 chromosome wheats has been carried out one would not expect all our varieties to have either 28 or 42 chromosomes: some should have 30, 32 or 40 (say). Although the chromosomes of only a comparatively small number of pure breeding forms have been examined it is unlikely that these intermediate numbers ever occur as they would probably show themselves by their irregular behaviour when crossed with other wheats.

Before concluding this section it will be useful to mention the method followed in obtaining the counts given in the preceding lists. In the case of a plant with less than 35 chromosomes the total number of chromosomes on the heterotype equatorial plate is readily found, and this gives the sum of the number of bivalents and the number of univalents; polar view counts at heterotype anaphase may give various results, as already explained, but if several are done it will always be found that some show two sets of 14 halves of bivalents, never less than 14. If 14 be the number of bivalents we then know at once the number of univalents, the sum of the two together being known, while the results so obtained should fit in with the other polar view counts and should be confirmed by the maximum number of splitting univalents observed at heterotype anaphase in side view.

For a plant with more than 35 chromosomes the sum of the univalents and bivalents is readily shown to be 21 from counts of the

heterotype equatorial plates. It is then necessary to find the minimum number of bivalents indicated by the polar view counts of segregation (*e.g.* counts such as 19—2—19, 18—3—18, 18—2—19, and so on, might be found; then 18 is the number of bivalents) of which as many as possible should be carried out; confirmation is provided by finding the maximum number of univalents observed at anaphase in side view.

In plants where irregularities are few little difficulty is experienced, but where irregularities are frequent considerable care is necessary, and as many counts as possible should be made before the results can be accepted with certainty. Longitudinal section can, in general, only give evidence upon the number of univalents seen in side view at heterotype anaphase, and for this purpose they are very useful. If 4 univalents are seen, then although there must be at least this number present it cannot be stated definitely that there are not more. Examination of a large number of cells at this stage will probably indicate, however, what the total number really is. Polar view counts made at heterotype metaphase and anaphase must be made from transverse sections, for it is only here that comparison with neighbouring sections can show with certainty what part or parts of a cell have been cut off. No count can be accepted as evidence unless it is quite clear; that is to say, unless the individual chromosomes are all distinct and no disturbing factor has been introduced such as displacement or cutting of chromosomes with the microtome knife.

When making observations many hundred cells are examined but very few of these will give reliable counts. This means of course that a large number of cells are rejected as not supplying reliable evidence, but no cell which seems not to conform with the results already obtained should be rejected until careful examination has revealed the reason for the discrepancy. Occasionally, for instance, in a cell at heterotype anaphase, one of the components of a bivalent chromosome may be displaced by the microtome knife and appear to be an extra univalent, but in a case such as this the evidence given by the cell can only be rejected if the reason for the discrepancy be clearly substantiated on other grounds.

As many counts as possible must be made of the segregation at heterotype anaphase; none can be accepted that is not quite clear, and all those accepted must be noted down. When all the observations are completed the evidence collected is considered, and if the chromosome number of the plant be not evident then the slides must be studied further until the reason for the ambiguity is known.

## THE FREQUENCY OF THE VARIOUS GAMETE CLASSES.

It is now proposed to discuss the significance of the observations recorded in Tables I and II, and to work out from them the frequency of the various gamete classes and the composition of the population resulting from random mating of the male and female.

It might be objected that loss of chromosomes does not occur in a sufficiently definite manner to justify us in using the results given in the tables either as evidence upon which to discuss the fate of lost chromosomes, as has been already done, or as data upon which to base calculations of the frequency of the gamete classes. It will be seen, however, that in Tables II (a) and II (c) counts made from different flowers of the same ear are in fairly good agreement; moreover, the general character of all the observations recorded in Tables I and II suggests that the chromosomes behave in a very definite way.

(1) *Loss of chromosomes at the Heterotype Division.*

If the chance of any one univalent chromosome being lost be  $n$ , and the number of univalents present in the plant be  $y$ , then the frequency of cells with 0, 1, 2 ...  $y$ , lost chromosomes are given by the terms in the expansion of

$$\{(1 - n) + n\}^y.$$

In plant A, for example, the frequency of cells with 0, 1, 2, 3 lost chromosomes will be

0	1	2	3
$(1 - n)^3$	$3n(1 - n)^2$	$3n^2(1 - n)$	$n^3$

and the value  $n = 0.33$  gives the frequencies

0	1	2	3
0.30	0.44	0.22	0.04

as given in Table I (a).

Similarly with plant B, where  $y = 4$ , the value  $n = 0.29$  gives the series of values shown in Table II (a).

In both cases it will be seen that the observed values for chromosome loss and the values found by assigning a special value to  $n$  agree fairly well; the deviation is not in fact greater than would be expected from the theory of random sampling. It therefore seems that loss of chromosomes at this division is purely a random one—the chance of any one chromosome being lost is the same, and if one has already been lost this does not affect the fate of the others, nor is there any tendency for all to be lost together.

(2) *Re-inclusion of lost chromosomes.*

In plant B some of the chromosomes previously lost are included in the homotype division (see p. 141), as shown by counts of their frequency at homotype metaphase. If  $x$  be the chance of any one chromosome being excluded from the homotype the chance of 0, 1, ... 4, being excluded will be given by the terms in the expansion of

$$\{(1 - x) + x\}^4.$$

The value  $x = 0.13$  gives the values indicated in Table II (c) and these again agree closely with the series of values observed, indicating that regain of chromosomes occurs at random, as was expected (see p. 142).

Subsequent regain occurs, in both plants, when the chromosomes taking part in the homotype division reach the poles, as they may meet there one or more of the chromosomes previously lost at the heterotype. The frequency with which chromosomes lost in the heterotype will still be lost at homotype telophase is given by the terms in the expansion of

$$\{(1 - n \overline{1 - m}) + n \overline{1 - m}\}^v,$$

where  $m$  is the chance of regain of any one. In the case of B and plants of similar behaviour  $x$  must be substituted for  $n$  in this expression.

The values assigned to  $m$  in the tables show good agreement with expectation (Tables I (e) and II (h)). Once again, regain of chromosomes previously lost seems to occur at random.

(3) *Loss of Univalents in the Homotype Division.*

Let  $p$  be the chance that any one of those univalents taking part in the division should get lost (not all take part as some are already lost).

The chance of any one univalent being present in the homotype division of a plant such as A is

$$(1 - n),$$

and, since in the homotype the univalents segregate at random, the chance that any one of those present should go to a particular pole is

$$\frac{1}{2},$$

therefore the chance that any one chromosome should be lost at a particular pole of the homotype is

$$p \cdot \frac{1 - n}{2},$$

and the frequency of cells with 0, 1, 2, ...  $y$  chromosomes excluded at



the end of the homotype division will be given by the terms in the expansion of

$$\{(1 - \frac{1}{2}p \overline{1-n}) + \frac{1}{2}p \overline{1-n}\}^v.$$

In the case of a plant such as B,  $x$  must be substituted for  $n$  in this expression.

In both cases very good agreement is obtained between observed and expected values upon assigning to  $p$  the "best" value (see Tables I ( $f$ ) and II ( $g$ )).

#### (4) *Random Segregation of Chromosomes in the Homotype.*

Except for the agreement between observed and expected values found in the last paragraph it is difficult to obtain definite evidence that the segregation of univalents in the homotype is truly a random one. With the object of obtaining evidence on this point counts were made in plant B of the frequencies with which 0 and 0, 0 and 1, 0 and 2, ..., etc. chromosomes were left outside the two nuclei formed at the end of the homotype division, that is at opposite poles of the same cell; it was thought that this might provide evidence on the relative frequency with which the chromosomes actually travelled to opposite poles.

The frequency of cells in which 0, 1, 2, 3 or 4 univalents take part in the homotype are given by the terms in the expansion of

$$\{x + (1-x)\}^4.$$

The frequency of the various chromosome losses are

0-0	$\{x + (1-p)(1-x)\}^4,$
0-1	$4(1-x)p\{x + (1-p)(1-x)\}^3,$
0-2	$6\frac{p^2}{2}(1-x)^2\{x + (1-p)(1-x)\}^2,$
0-3	$4\frac{p^3}{4}(1-x)^3\{x + (1-p)(1-x)\},$
0-4	$\frac{1}{8}p^4(1-x)^4,$
1-1	$6\frac{p^2}{2}(1-x)^2\{x + (1-x)(1-p)\}^2,$
1-2	$4\cdot\frac{3}{4}p^3(1-x)^3\{x + (1-x)(1-p)\},$
1-3	$\frac{1}{2}p^4(1-x)^4,$
2-2	$\frac{3}{8}p^4(1-x)^4.$

Agreement between observed and expected values (Table II ( $f$ )) is not very close, and it is evident that the number of observations made was insufficient, but unfortunately it does not seem likely at present

that a greater number would be possible on any one plant. However, the results are on the whole favourable to the assumption that random segregation is taking place, an assumption that appears probable on *a priori* grounds. It is hoped that further evidence on this point will be obtained later.

(5) *The Frequencies of the Gamete Classes.*

From the results obtained in the preceding four sections it is possible to calculate the frequencies of the microspores having 0, 1, 2, ... univalents present in the nucleus. In a plant such as A the chance of any one univalent being included in a microspore nucleus is

$$\frac{(1-n)}{2} \cdot (1-p) + \frac{nm}{2} = \frac{1-n-p+pn+mn}{2}.$$

And the frequencies with which these nuclei will possess 0, 1, 2, ...  $y$  univalents are given by the terms in the expansion of

$$\left\{ \frac{1+n+p-pn-mn}{2} + \frac{1-n-p+pn+mn}{2} \right\}^y.$$

In plants such as B  $x$  must be substituted for  $n$  in this expression.

The frequencies of the microspore classes in A and B are given in Tables I ( $h$ ) and II ( $q$ ) respectively.

The correctness of these results (Tables I ( $h$ ) and II ( $q$ )) depends upon the validity of the conclusions drawn above—that loss and regain of chromosomes occurs at random, and that the segregation of the univalents in the homotype is a random one. The results given are sufficient to justify the first of these conclusions, and the second also, can probably be accepted (see previous section).

The evidence that the lost chromosomes present in the microspores degenerate has been discussed (pp. 142–143). If this evidence be accepted, and we also assume that the abortion of certain pollen grains has not been selective (see p. 143), then the frequencies of the gamete classes are the same as the frequencies of the microspore classes. These two assumptions, more especially the second, can only be accepted with reserve, but though further work will probably make necessary some modification, it is unlikely that the respective frequencies of the gamete and microspore classes will be found to differ very widely.

In the reduction divisions of the  $F_1$  the 14 *turgidum* chromosomes pair with 14 of those of the *vulgaris* parent, and, as far as can be determined, the components of the 14 bivalents so formed segregate quite normally; moreover, in the five plants noted above (p. 143) as having

28 chromosomes the reduction divisions are quite normal. It has therefore been concluded that random segregation of these two sets of chromosomes occurs, in the usual manner. Furthermore, not only does the fact that pairing between them takes place suggest that these 14 *vulgare* chromosomes are similar in nature to the *turgidum* chromosomes, but since all the above-mentioned five plants were fertile it would seem that this similarity is sufficient for one or more of one set to replace those of the other without interfering in any essential manner with development.

Let the complete set of *turgidum* chromosomes, or their *vulgare* homologues, be denoted by  $X$ , and let the remaining 7 *vulgare* chromosomes be denoted by  $A, B, C, \dots G$ . Then the gametes of the  $F_1$  all contain  $X$  and in addition may contain any selection of from 0 to 7 of the univalents.

Let  $p_0$  be the frequency of gametes having  $X$  alone,  
 $p_1$  the frequency of those having  $A$ , or  $B$ , or  $C \dots$ , or  $G$ ,  
 $p_2$  the frequency of those having  $A + B, A + C, A + D, B + C$ , or ...,  
 $p_3 \dots \dots \dots$   
 $p_7$  the frequency of those having  $A + B + C + D + E + F + G$ .

The assumption that the frequencies of  $X + A, X + B, X + C$ , etc., are all  $p_1$ , and the frequencies of  $X + A + B$ , etc., are all  $p_2$ , and so on, is justified as all the evidence already given points to the fact that loss of these chromosomes and, probably, their segregation in the homotype has taken place at random.

The total population of the  $F_1$  gametes may therefore be written

$$\begin{aligned}
 p_0 \cdot X + p_1 \Sigma (X + A) + p_2 \Sigma (X + A + B) + p_3 \Sigma (X + A + B + C) \\
 + p_4 \Sigma (X + A + B + C + D) + p_5 \Sigma (X + A + B + C + D + E) \\
 + p_6 \Sigma (X + A + B + C + D + E + F) + p_7 (X + A + B + C + D \\
 + E + F + G).
 \end{aligned}$$

The actual values of  $p_0, p_1, \dots p_7$  can be found from counts of the chromosome loss at reduction, and, subject to the reservations made on p. 143, we can accept with some confidence the results so obtained for the frequencies of the different classes of male gamete. For instance, in the case of plant B we have from Table II ( $g$ ),

$$\begin{aligned}
 p_0 = 0.207, & \quad 4p_1 = 0.399 \text{ (the sum of the frequencies of the 4 classes} \\
 & \quad X + A, X + B, X + C, X + D \text{ is equal to } 0.399) \\
 6p_2 = 0.288, & \quad 4p_3 = 0.093, & \quad p_4 = 0.011,
 \end{aligned}$$

giving, for the population of male gametes in this plant,

$$0.207X + 0.099\Sigma(X + A) + 0.048\Sigma(X + A + B) \\ + 0.024\Sigma(X + A + B + C) + 0.011\Sigma(X + A + B + C + D).$$

The population of the male gametes from plant A is given in Table I (*h*).

THE COMPOSITION OF THE POPULATION RESULTING FROM SELF-  
FERTILISATION OF THE PLANTS UNDER CONSIDERATION.

(1) If the frequencies of the gamete classes are the same in the female as in the male, and if random mating between them occur, then the resulting population would be given by the square of the expression obtained for the population of the male gametes. In the case of the plant B the progeny population, grouped according to the number of chromosomes they possess, would be:

No. of  
chromosomes

$$34 \quad p_0^2 X^{2*}$$

$$35 \quad 2p_0p_1 \Sigma(X^2 + A)$$

$$36 \quad p_1^2 \Sigma(X^2 + A^2) + 2p_0p_2 \Sigma(X^2 + A + B) + 2p_1^2 \Sigma(X^2 + A + B)$$

$$37 \quad 2p_1p_2 \{\Sigma(X^2 + A^2 + B) + 3\Sigma(X^2 + A + B + C)\} \\ + 2p_0p_3 \Sigma(X^2 + A + B + C)$$

$$38 \quad 2p_0p_4 (X^2 + A + B + C + D) + 2p_1p_3 \{\Sigma(X^2 + A^2 + B + C) \\ + 4(X^2 + A + B + C + D)\} + p_2^2 \{\Sigma(X^2 + A^2 + B^2) \\ + 2\Sigma(X^2 + A^2 + B + C) + 6(X^2 + A + B + C + D)\}$$

$$39 \quad 2p_1p_4 \Sigma(X^2 + A^2 + B + C + D) + 2p_2p_3 \{\Sigma(X^2 + A^2 + B^2 + C) \\ + 3\Sigma(X^2 + A^2 + B + C + D)\}$$

$$40 \quad 2p_2p_4 \Sigma(X^2 + A^2 + B^2 + C + D) + p_3^2 \{\Sigma(X^2 + A^2 + B^2 + C^2) \\ + 2\Sigma(X^2 + A^2 + B^2 + C + D)\}$$

$$41 \quad 2p_3p_4 \Sigma(X^2 + A^2 + B^2 + C^2 + D)$$

$$42 \quad p_4^2 (X^2 + A^2 + B^2 + C^2 + D^2)$$

\*  $X$  here represents a single set of the 17 components of the bivalents of this plant.

Where an expression such as  $X^2 + A^2 + B^2 + C$  means that the 17 chromosomes  $X$ , and chromosomes  $A$  and  $B$ , are present in the bivalent condition, and  $C$  in the univalent condition; while  $\Sigma(X^2 + A^2)$  means the four classes  $X^2 + A^2$ ,  $X^2 + B^2$ ,  $X^2 + C^2$ ,  $X^2 + D^2$ ; similarly with the other terms in the expression.

Of these zygotes those with 34 chromosomes have 17 bivalents, those

with 35 have 17 bivalents and one univalent, those with 36 have 18 bivalents or 17 bivalents and 2 univalents, and so on, the combinations found being:

	No. of bivalents	No. of univalents
(1)	17	0
(2)	17	1
(3)	17	2
(4)	17	3
(5)	17	4
(6)	18	0
(7)	18	1
(8)	18	2
(9)	18	3
(10)	19	0
(11)	19	1
(12)	19	2
(13)	20	0
(14)	20	1
(15)	21	0

It will be evident that similar combinations result from self-fertilisation of the  $F_1$  or of any plant possessing univalent chromosomes. We have already seen, however, that many of these combinations are not found, and it has been pointed out that the number of plants investigated by Kihara and myself, especially when taken in conjunction with other evidence, is sufficient to warrant the belief that they do not exist. We are therefore led to the conclusion that certain of the possible chromosome combinations are non-viable. The combinations that fail to develop—Nos. (1) to (4), (6) to (8), (10), (11) and (13) in the above list—are those which do not possess all four of the chromosomes *A*, *B*, *C* and *D* at least once. This means, if the assumptions made above are correct, that a complete set of the 7 univalents is necessary for development. It would follow also that a plant with more than 35 chromosomes would never have offspring with fewer chromosomes than itself. Kihara actually found this to be the case in an instance in which he was able to find the chromosome number of 9 of the offspring of a 38 chromosome plant.

In the same way we can arrive at the population from a plant with less than 35 chromosomes; the expression obtained (p. 151) for plant B will apply equally to a plant with 14 bivalents and 4 univalents if *X* here represent the 14 components of the bivalents and *A*, *B*, *C*, *D*, as before, the 4 univalents. Here it is the zygotes with more than 14 bivalents which fail to develop; that is to say, development is not interfered with by the addition of one or more of the 7 *vulgare* chromosomes to the 14 bivalents so long as these additional chromosomes are present only in the univalent condition—one of each kind. In the case of a plant with

less than 35 chromosomes, therefore, it would follow that none of its offspring have more chromosomes than it has itself. Evidence for this is supplied by Kihara's results.

Before drawing further conclusions attention must be drawn to the two assumptions made at the beginning of this section (p. 151), namely that the population of female gametes is identical with that of the male, and that random mating between male and female takes place. If these two assumptions are even approximately true the above reasoning will apply—the actual chromosome combinations resulting from self-fertilisation would be the same though the relative frequency of their occurrence would be different. There are, however, two extreme cases of divergence from the conditions implied by these assumptions which must be considered.

(2) First, suppose that the frequency of the female gamete classes is the same, or approximately the same, as those of the male, but that selective mating occurs in such a way that in a plant with less than 35 chromosomes only male gametes with 14 chromosomes effect fertilisation, and in plants with more than 35 chromosomes only those male gametes possessing 21 chromosomes. This could result from differential rates of growth of pollen tubes or from some other cause; it would explain why only plants with either 14 bivalents, and no more, or with a complete set of 7 univalents, are found. It would also explain the fact that plants with 42 chromosomes appear in the  $F_2$  generation from *turgidum*  $\times$  *vulgare* crosses with much greater frequency than is to be expected on the theory of random mating between gametes with the same class frequencies—about once in a thousand instead of once in a million<sup>1</sup>.

(3) Secondly, suppose that random mating or an approximation thereto occurs, but that in a plant with less than 35 chromosomes only egg cells with 14 chromosomes are functional, and in a plant with more than 35 chromosomes only egg cells with 21 chromosomes.

This possibility can probably be ruled out. Egg cell development will be described in a later paper, but in the meantime it may be stated that the evidence to hand suggests first that the frequency with which univalents are included in the nuclei of the tetrads does not differ widely from the corresponding frequency found in pollen development (compare, for example, Tables II (*g*) and II (*p*)), and secondly that degeneration of three of the cells of the tetrads has not been selective. This being the case it is obvious that the proportion of non-functional egg cells in the  $F_1$  would have to be very high if only those with 14 or

<sup>1</sup> This estimate is of course only approximate.

21 chromosomes function, and this is not the case for the proportion of grains set on these plants may be as high as 0.42. In addition we have the fact that non-functional egg cells are often not distributed at random up the spike.

#### ✓ STERILITY.

It has already been mentioned (p. 130) that in the crosses which form the subject of the present paper—crosses between *T. turgidum* with 28 chromosomes and *T. vulgare* with 42—the  $F_1$  and many of the plants of the later generations are partially sterile. That is to say, the two lowest flowers of the spikelet do not always set grain, and the grains set are arranged in an irregular manner up the spike. All gradations are found between quite plump and very shrivelled grains. Also, many of the grains fail to germinate and many of the plants die away in the young stages.

In view of the conclusions reached in the last section these facts are naturally of interest, and though it is proposed to reserve for a later paper a fuller discussion of the problem of sterility, and of the conclusions reached by Kihara and by Sax on this problem, it seems proper to consider certain aspects of the situation here.

Sax (1922, p. 536) seeks to explain partial sterility in wheat crosses by supposing that gametes with 17 or 18 chromosomes fail to function. The fact that in plant B (see Table II (*g*)) the sum of the frequencies of the 17 and 18 chromosome gametes equals 0.61, while the proportion of empty pollen grains is only 0.22, may seem to make this supposition unlikely. It must, however, be admitted, as East (1921, p. 333) and Sax (1922, p. 523) have pointed out, that apparent morphological perfection is no certain criterion for the ability of a pollen grain to function. But in any case Sax's hypothesis is not sufficient to account for the fact already pointed out that plants with more than 35 chromosomes all have a complete set of 7 univalents while plants with less than 35 chromosomes do not have more than 14 bivalents.

Kihara (1921) discusses at length the failure of all the expected chromosome combinations to appear in the  $F_2$  and succeeding generations. He appears to take it as self-evident that in any plant the number of the bivalents is equal to the number of chromosomes transmitted by one of the parent gametes. This being so, then, we see from the fact that only combinations such as 18 bivalents + 3 univalents, or 14 bivalents + 3 univalents, appear, that one at least of the parent gametes always contains either 14 or 21 chromosomes. He therefore argues that the

union of two gametes only produces a viable zygote when this condition is fulfilled; if neither female nor male gamete is so constituted, then either the embryo formed from their union does not develop, or the grain does not germinate, or the young plant does not live. This theory cannot be accepted. The number of bivalents found in a plant is not necessarily equal to the number of chromosomes possessed by one of the parent gametes. Also, a zygote formed by the union of gametes both possessing 19 chromosomes may be identical, as far as its chromosome content is concerned, with one formed by the union of gametes with 17 and 21 chromosomes respectively; yet according to Kihara's theory the second is viable and the first is not.

From the work which forms the subject of the present paper it was concluded in the preceding section (pp. 151-154) that there are two possible explanations of the fact that not all the expected chromosome combinations are found in the  $F_2$  generation of the crosses we are considering. The first of these theories—that random mating between gametes with the same class frequencies gives certain chromosome combinations that are non-viable—is attractive by reason of its possible bearing on the problem of sterility, but it cannot yet be definitely accepted. From the expression given on p. 151 it is readily seen that the total frequency of non-viable zygotes would be, in the case of plant B,

$$p_0^2 + 8p_0p_1 + 16p_1^2 + 12p_0p_2 + 48p_1p_2 + 8p_0p_3 \\ + 24p_1p_3 + 30p_2^2 + 24p_2p_3 + 4p_3^2,$$

and this expression is equal to 0.91 if for  $p_0, p_1, p_2, p_3, p_4$ , we substitute the values found. Though there is no definite evidence to hand on this point my impression is that the proportion of grains which fail to produce mature plants is rarely, if ever, as high as this. Also there is the fact already mentioned (p. 153) that plants with 42 chromosomes probably appear in  $F_2$  with greater frequency than is to be expected from this theory.

#### SUMMARY AND CONCLUSIONS.

The  $F_1$  from a cross between *T. turgidum*, with a haploid chromosome number of 14, and *T. vulgare*, with 21 for haploid number, contains 35 chromosomes; at reduction 14 of those from one parent pair with 14 from the other parent leaving 7 chromosomes unpaired. In the  $F_2$  and later generations unpaired, univalent, chromosomes appear, and a detailed description of their behaviour during the reduction divisions has been given. Throughout these divisions the behaviour of the bivalents



is normal, but that of the univalents is quite different. After diakinesis the latter lag behind the others and do not usually reach the equatorial plate until the components of the bivalents are on their way to the poles. Arrived at the equatorial plate the univalents split longitudinally and the halves pass to opposite poles, but may arrive too late to be included in the daughter nuclei. In the homotype division those that were not lost segregate at random to opposite poles, but here again they are generally later than the chromosomes that were paired and may fail to be included in the daughter nuclei, as before. The chromosomes that were lost in the heterotype may, some of them (plant B), be included by the homotype spindle and take part in the division, or may all still lie outside the spindle at homotype metaphase (plant A); in either case many of them lie near the poles of the spindle, are met there by the chromosomes that took part in the division, and are included in the nucleus there formed. Chromosomes lost at the heterotype division undergo successively—during the stages that follow—condensation, vacuolation (during interkinesis), condensation (at homotype prophase), lengthening into thin threads (homotype anaphase), condensation (homotype telophase); these changes have an exact parallel in the changes undergone by the chromosomes taking part in the homotype division. Loss, and subsequent regain of chromosomes when this occurs, both happen quite at random, and the same is probably the case with segregation of the univalents in the homotype division.

As the microspore enlarges the lost chromosomes begin to degenerate, but whether all finally do so cannot be stated as the study of pollen development has not yet been completed. A certain proportion of empty pollen is found, but the reason for this is not yet definitely known.

The frequencies of microspores possessing the various possible chromosome combinations have been calculated. It has been pointed out that this result probably gives us an approximation to the frequencies of the various classes of male gamete, and there is little doubt that when the study of pollen development has been completed the frequencies of these latter can be found accurately. This result is of the greatest importance, for it is only with this knowledge that a detailed study of the genetics and cytology of these crosses can be attempted.

In the  $F_2$  and later generations plants with less than 35 chromosomes all have 14 bivalents—never more, while in plants with more than 35 chromosomes the sum of the number of bivalents and the number of univalents is always equal to 21. Two theories have been put forward to account for these facts, and while it is felt that there is considerable

evidence that one or other of these theories must be correct it is nevertheless realised that there are sufficient possible complexities to make further work necessary before either can be definitely accepted.

In the first theory it has been assumed that the frequencies of the gamete classes are the same in male and female, and that random mating takes place; in this way an expression has been obtained for the composition of the population arising from self-fertilisation of plants with univalent chromosomes. Some of the combinations thereby found never actually occur; this may be the reason for the often found failure of grains to germinate and for the early death of some of the young plants. Reasons have been given, however, for suspecting that this theory may not hold in the form stated.

In a second theory it has been supposed that the frequencies of the male and female gamete classes are the same, or approximately the same, but that in a plant with less than 35 chromosomes only male gametes with 14 chromosomes function, and in plants with more than 35 only those with 21. At present there are no facts known which contradict this theory.

It will be possible to discriminate between these two theories by further work.

From the evolutionary viewpoint the problem of the genetic relationship between wheats of the Emmer group and those of the *vulgare* group is one of great interest, for it is at present difficult to see how species with 42 chromosomes can have arisen from one with 28, and it seems that this must at one time have occurred. An extended series of breeding experiments will be necessary for the elucidation of this problem but it is already interesting to note that in the reduction divisions of the  $F_1$  from the species cross all the 14 chromosomes from one parent pair with 14 of those from the other, and that the segregation of these 28 chromosomes takes place in normal fashion. Our knowledge of chromosome behaviour is, as yet, insufficient for us to conclude definitely from these facts that the two sets of 14 chromosomes are essentially similar in nature, but it would certainly appear that such is the case. Pairing between the parental chromosomes in a species cross need not necessarily take place; we may cite, for example, the cross *Crepis setosa*  $\times$  *C. capillaris* (Collins and Mann, 1923) in the  $F_1$  of which none of the chromosomes pair, and other instances are well known.

## APPENDIX

In the preceding discussion the possibility that univalents may pass undivided to the poles of the heterotype spindle (see p. 139) has been neglected. If we assume that failure to divide, if it occur, takes place at random, and that the chance that any one univalent should not divide in the heterotype be  $q$ , then the frequencies with which  $0, 1, \dots y$  univalents would be lost in this division are given by the terms in the expansion of

$$\{(1 - n \overline{1 - q}) + (n \overline{1 - q})\}^y,$$

where  $n$  is here equal to the chance of loss of a daughter half of any chromosome that has divided. The value of  $n(1 - q)$  can thus be found and will clearly be equal to that found in the previous calculations (p. 146) for  $n$ . Let this value be  $K_1$ .

The value of  $m$  (see p. 147) will clearly be unaltered.

The expression for loss of univalents in the homotype division will be

$$\{(1 - \frac{1}{2}p \overline{1 - q} \overline{1 - n}) + (\frac{1}{2}p \overline{1 - q} \overline{1 - n})\}^y$$

and the value of  $p(1 - q)(1 - n)$  can be found, being equal to the value previously found for  $p(1 - n)$  (see pp. 147-148). Let this value be  $K_2$ .

The expression for the frequencies of the microspore classes will be

$$\left\{ \frac{1 + p(1 - q)(1 - n) + n(1 - q)(1 - m)}{2} + \frac{1 - p(1 - q)(1 - n) - n(1 - q)(1 - m)}{2} \right\}^y$$

instead of

$$\left\{ \frac{1 + p(1 - n) + n(1 - m)}{2} + \frac{1 - p(1 - n) - n(1 - m)}{2} \right\}^y,$$

and each of these expressions is equal to

$$\left\{ \frac{1 + K_2 + K_1(1 - m)}{2} + \frac{1 - K_2 - K_1(1 - m)}{2} \right\}^y.$$

The frequencies of the microspore classes will therefore be unaltered if univalents sometimes segregate at random in the heterotype, and divide longitudinally in the homotype and pass to the poles without being lost.

If the actual values of  $p$  and  $n$  be required these can only be found from additional observations. We do know, however, that

$$n(1 - q) = K_1, \text{ where } K_1 \text{ is constant,}$$

and that

$$p(1 - q)(1 - n) = K_2, \text{ where } K_2 \text{ is constant,}$$

and it follows that the relation between  $p$  and  $q$ , or between  $n$  and  $q$ , is parabolic, for

$$pq = p(1 - K_1) - K_2$$

and

$$nq = n - K_1.$$

These two equations enable us to give a maximum value to  $q$ , since  $n$  and  $p$  must each be positive and not greater than unity. The probability is, of course, that  $q$ ,  $p$  and  $n$  are constant, but the evidence presented does not allow us to conclude this finally.

In conclusion I wish to thank Professor Biffen for providing me with the Rivet  $\times$  Iron  $F_2$ , and Mr Engledow for the Rivet  $\times$  Mesopotamian *vulgare*  $F_2$ , as well as for his helpful criticisms and suggestions while the paper was being prepared.

TABLE I.

*Frequency of Chromosome Loss in Plant A.*

(a) *Loss from heterotype.*

Count made at interkinesis.

No. of lost chromosomes	No. of cells	Propn. of cells	Expectation if $n = 0.33$
0	22	0.32	0.30
1	27	0.39	0.44
2	19	0.27	0.22
3	1	0.02	0.04

(b) *Number present at homotype metaphase.*

Count made at about the stage of Fig. 37.

No. of lost chromosomes	No. of cells	Propn. of cells
0	20	0.37
1	24	0.44
2	9	0.17
3	1	0.02

(c) *Present at homotype anaphase.*

Count made, at about the stage of Figs. 41 or 46, of those chromosomes lost in the heterotype and not later reincluded.

No. of lost chromosomes	No. of cells	Propn. of cells
0	20	0.77
1	6	0.23
2	0	0.00
3	0	0.00

TABLE I (continued).

(d) *Present at homotype telophase.*

Count made at about the stage of Fig. 51.

No. of lost chromosomes	No. of cells	Propn. of cells
0	18	0.78
1	4	0.18
2	1	0.04
3	0	0.00

(e) *Total count of chromosomes lost in the heterotype without being subsequently regained.*

The sum of the two preceding counts.

No. of lost chromosomes	No. of cells	Propn. of cells	Expectation if $m=0.80$
0	38	0.78	0.78
1	10	0.20	0.20
2	1	0.02	0.02
3	0	0.00	0.00

(f) *Loss at the homotype.*

Count made at homotype telophase, about the stage of Fig. 51.

No. of lost chromosomes	No. of cells	Propn. of cells	Expectation if $p=0.66$
0	26	0.48	0.47
1	22	0.41	0.41
2	6	0.11	0.12
3	0	0.00	0.01

(g) *Present in cells of tetrad.*

Count made at the stage of Fig. 55.

No. of densely staining chromosomes	No. of cells	Propn. of cells
0	39	0.51
1	31	0.40
2	7	0.09
3	0	0.00
No. of vacuolated chromosomes	No. of cells	Propn. of cells
0	57	0.74
1	19	0.25
2	1	0.01
3	0	0.00

(h) *Frequency of gamete classes.*

The frequency of microspores having 0, 1, 2 or 3 univalents present in the nucleus is given by the terms in the expansion of

$$\left\{ \frac{1+n+p-pn-mn}{2} + \frac{1-n-p+pn+mn}{2} \right\}^2$$

or, since  $n=0.33$ ,  $p=0.66$ ,  $m=0.80$ ,

$$(0.76+0.24)^2.$$

∴ we have

No. of univalents present in microspore nucleus	Frequency
0	0.43
1	0.42
2	0.14
3	0.01

It has been assumed that the frequency of the gamete classes is the same as that of the microspore classes.

TABLE II.

*Frequency of Chromosome Loss in Plant B.*(a) *Loss at the heterotype.*

Count made at interkinesis.

No. of lost chromosomes	No. of cells		Propn. of cells		Propn. of cells (total count)	Expectation if $n=0.29$
	(1)	(2)	(1)	(2)		
0	9	11	0.28	0.20	0.23	0.25
1	15	25	0.47	0.45	0.46	0.42
2	6	13	0.19	0.24	0.22	0.25
3	2	5	0.07	0.09	0.08	0.07
4	0	1	0.00	0.02	0.01	0.01

The results given under (1) and (2) were obtained from different flowers of the same ear.

(b) *Present at homotype prophase.*

Count made at about the stage of Figs. 32 and 33.

No. of lost chromosomes	No. of cells	Propn. of cells
0	8	0.31
1	11	0.42
2	5	0.19
3	2	0.08
4	0	0.00

(c) *Present at homotype metaphase.*

Count made at about the stage of Fig. 37.

No. of lost chromosomes	No. of cells		Propn. of cells		Propn. of cells (total count)	Expectation if $x=0.13$
	(1)	(2)	(1)	(2)		
0	15	35	0.58	0.57	0.57	0.56
1	8	21	0.31	0.34	0.33	0.35
2	3	5	0.11	0.09	0.10	0.08
3	0	0	0.00	0.00	0.00	0.01
4	0	0	0.00	0.00	0.00	0.00

The results given under (1) and (2) were obtained from different flowers of the same ear.

(d) *Present at early homotype anaphase.*

Count made at about the stage of Fig. 39.

No. of lost chromosomes	No. of cells	Propn. of cells
0	11	0.58
1	5	0.26
2	3	0.16
3	0	0.00
4	0	0.00

(e) *Lost univalents present at homotype telophase.*

Counts made at about the stage of Fig. 51. The frequencies of (1) loss at the homotype, and (2) chromosomes lost in the heterotype and not regained, are given.

(1)	No. of chromosomes lost at the homotype	No. of cells	Propn. of cells
	0	32	0.53
	1	20	0.33
	2	8	0.14
	3	0	0.00
	4	0	0.00

TABLE II (continued).

(2)	No. of chromosomes lost at the heterotype	No. of cells	Propn. of cells
	0	50	0.83
	1	10	0.17
	2	0	0.00
	3	0	0.00
	4	0	0.00
(3)	Total no. of lost chromosomes in cell	No. of cells	Propn. of cells
	0	26	0.43
	1	22	0.37
	2	12	0.20
	3	0	0.00
	4	0	0.00

(f) *Homotype segregation.*

Counts made at stage of Fig. 51 of cells with the various possible chromosome losses.

Nos. of lost chromosomes	No. of cells	Propn. of cells	Calculated propn.
0-0	12	0.18	0.24
0-1	35	0.54	0.42
0-2	10	0.15	0.135
0-3	1	0.02	0.02
0-4	0	0.00	0.00
1-1	5	0.08	0.135
1-2	2	0.03	0.06
1-3	0	0.00	0.00
2-2	0	0.00	0.00

The calculated proportion, given in the last column, is obtained by putting  $p=0.35$  and  $x=0.13$ , and assuming that segregation in the homotype takes place at random.

(g) *Total count of loss at the homotype.*

Obtained by adding the count from (e) (1) to that given by the results of the last table.

No. of lost chromosomes	No. of cells (from Tables (e) and (f))	Propn. of cells	Expectation if $p=0.35$
0	$32 + 70 = 102$	0.54	0.53
1	$20 + 47 = 67$	0.35	0.37
2	$8 + 12 = 20$	0.10	0.10
3	$0 + 1 = 1$	0.01	0.00
4	$0 + 0 = 0$	0.00	0.00

(h) *Total count of chromosomes lost in the heterotype without being subsequently regained.*

Obtained by adding the results given in (e) to those of a separate count.

No. of lost chromosomes	No. of cells	Propn. of cells	Expectation if $m=0.66$
0	72	0.80	0.82
1	17	0.19	0.18
2	1	0.01	0.00
3	0	0.00	0.00
4	0	0.00	0.00

(i) *Lost chromosomes present in the cells of the tetrads.*

Count made at the stage of Fig. 55.

No. of lost chromosomes	No. of cells	Propn. of cells
0	70	0.52
1	48	0.35
2	18	0.13
3	0	0.00
4	0	0.00

TABLE II (*continued*).(k) *Present in microspores, stage 1.*

Count made at the stage of Figs. 69 and 70.

No. of densely staining chromosomes	No. of cells	Propn. of cells
0	40	0.69
1	16	0.27
2	2	0.04
3	0	0.00
4	0	0.00
No. of vacuolated chromosomes	No. of cells	Propn. of cells
0	42	0.72
1	14	0.24
2	2	0.04
3	0	0.00
4	0	0.00
Total no. of lost chromosomes in cell	No. of cells	Propn. of cells
0	28	0.48
1	22	0.38
2	8	0.14
3	0	0.00
4	0	0.00

(l) *Present in microspores, stage 2.*

Count made at the stage of Figs. 71 and 72.

No. of densely staining chromosomes	No. of cells	Propn. of cells
0	32	0.64
1	14	0.28
2	4	0.08
3	0	0.00
4	0	0.00
No. of vacuolated chromosomes	No. of cells	Propn. of cells
0	46	0.92
1	4	0.08
2	0	0.00
3	0	0.00
4	0	0.00
Total no. of lost chromosomes in cell	No. of cells	Propn. of cells
0	28	0.56
1	18	0.36
2	4	0.08
3	0	0.00
4	0	0.00

(m) *Present in microspores, stage 3.*

Count made at the stage of Fig. 73.

No. of densely staining chromosomes	No. of cells	Propn. of cells
0	33	0.66
1	13	0.26
2	3	0.06
3	1	0.02
4	0	0.00



TABLE II (continued).

(n) *Megaspore mother cell; loss at heterotype.*

Count made at interkinesis.

No. of lost chromosomes	No. of cells
0	0
1	1
2	1
3	1
4	1

(o) *Megaspore mother cell; loss at homotype.*

Count made at 4-cell stage. Compare with Table II (g), the count for pollen mother cells.

No. of lost chromosomes	No. of cells	Propn. of cells
0	6	0.50
1	3	0.25
2	1	0.08
3	2	0.17
4	0	0.00

(p) *Frequency of gamete classes.*

The frequencies of microspores having 0, 1, 2, 3 or 4 univalents present in the nucleus are given by the terms in the expansion of

$$\left\{ \frac{1+x+p-px-mx}{2} + \frac{1-x-p+px+mx}{2} \right\}^4,$$

or, since  $x=0.13$ ,  $p=0.35$ ,  $m=0.66$ ,

$$(0.675+0.325)^4,$$

we have

No. of univalents present in microspore nucleus	No. of chromosomes present in microspore nucleus	Frequency
0	17	0.207
1	18	0.399
2	19	0.288
3	20	0.093
4	21	0.011

and if we assume that the gamete classes have the same frequencies we have

$$\begin{array}{ll} p_0=0.207 & \text{giving } p_0=0.207 \\ 4p_1=0.399 & p_1=0.099 \\ 6p_2=0.288 & p_2=0.048 \\ 4p_3=0.093 & p_3=0.024 \\ p_4=0.011 & p_4=0.011 \end{array}$$

Two counts of sterile pollen gave

Good	Bad
520	141
429	122

Percentage of sterile pollen = 21.6.

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The following figures were drawn with the aid of a camera lucida. A 1/12 inch objective and No. 8 ocular were used throughout. Magnification = 1500 diameters.

*u.v.* = univalent.

FIGS. 1-77. Reduction in the microspore mother cells.

FIGS. 1-12. Heterotype metaphase.

FIGS. 13-22. Heterotype anaphase and telophase.

Fig. 14. In part a reconstruction, most of the chromosomes of the lower daughter nucleus being present in the adjacent section.

FIGS. 23-31. Heterotype telophase and interkinesis.

Fig. 25. The upper cell has been cut and the greater part of it is in the next section.

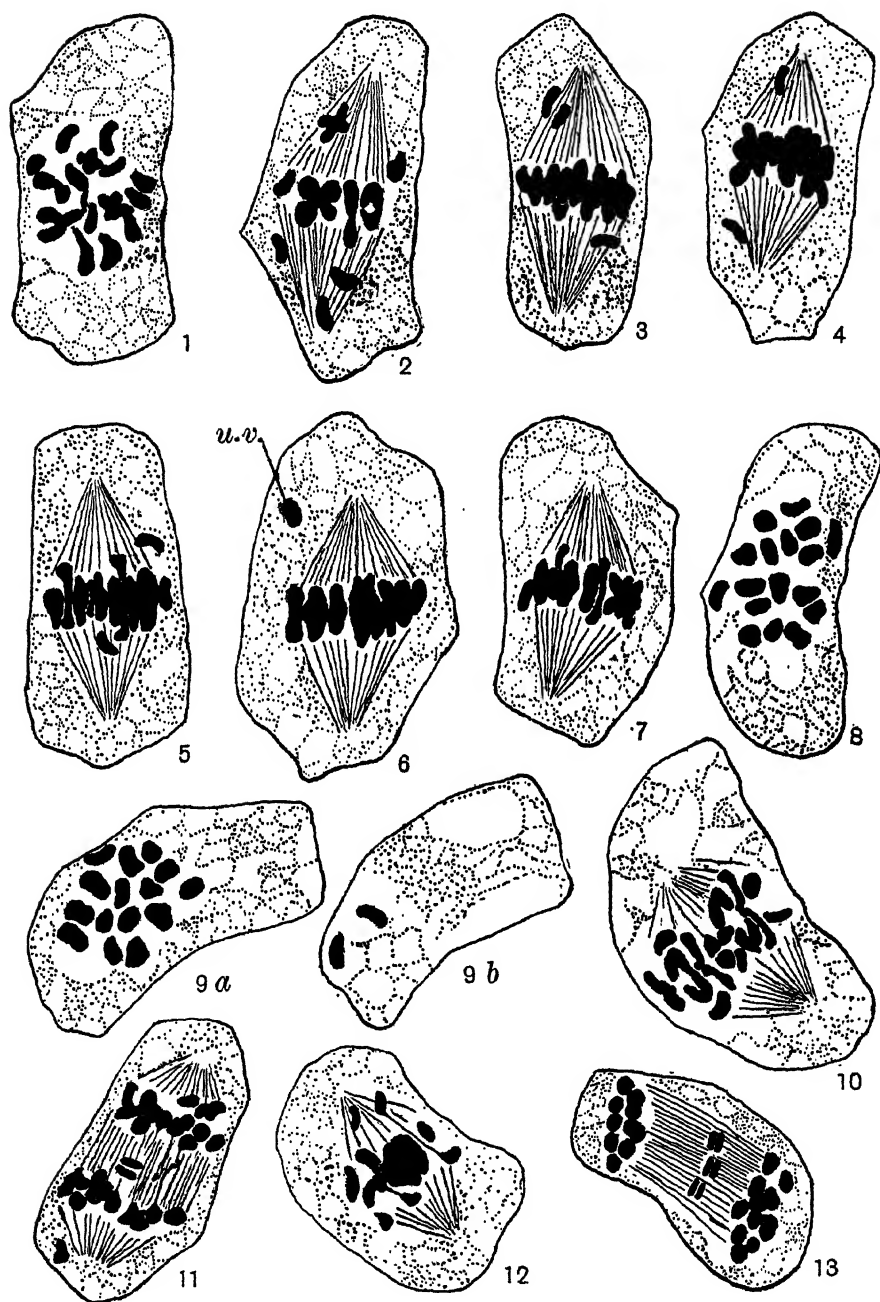
FIGS. 32-46. Homotype; prophase to anaphase.

FIGS. 47-58. Homotype anaphase and telophase.

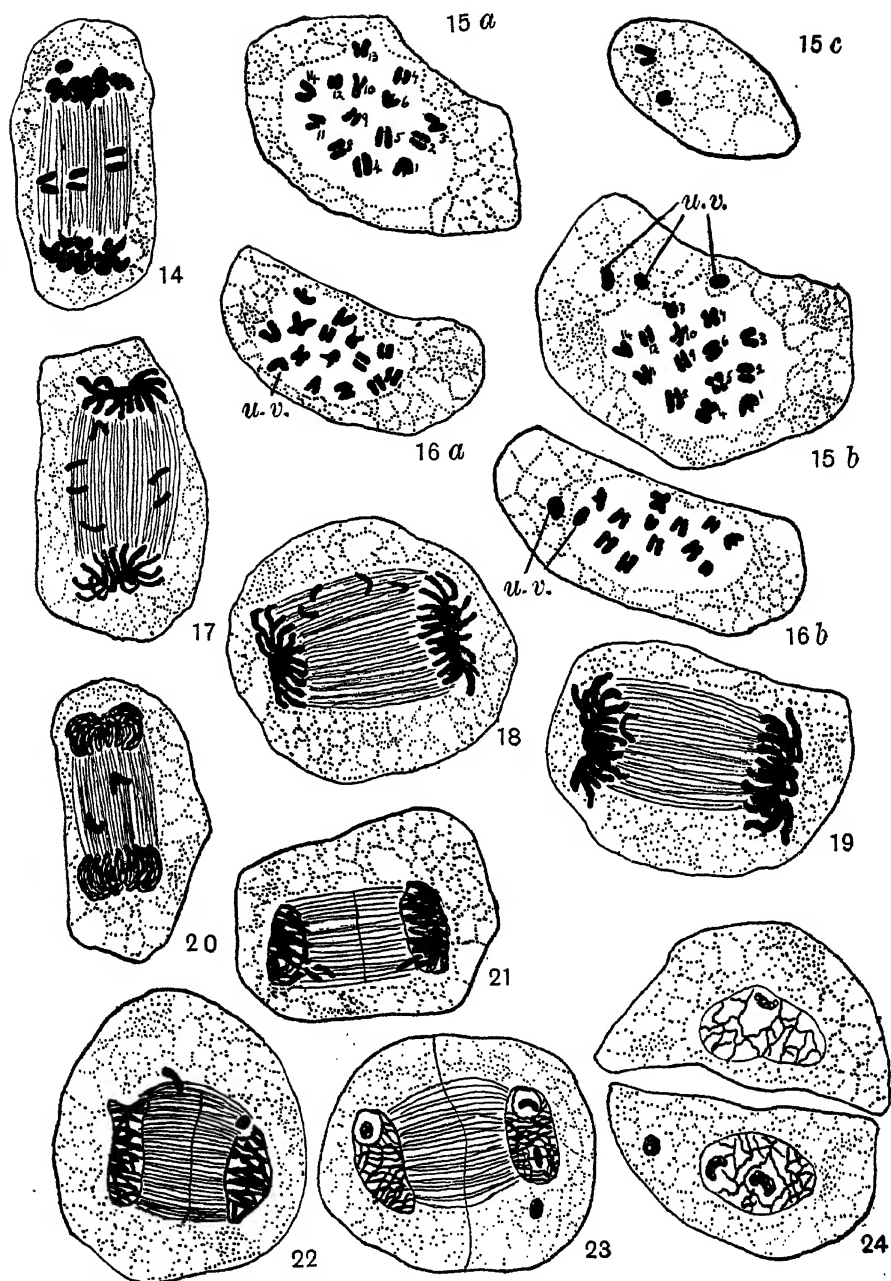
FIGS. 59-68. Heterotype irregularities.

Fig. 64. Some cytoplasm has been cut from this cell but the chromosomes were undisturbed.

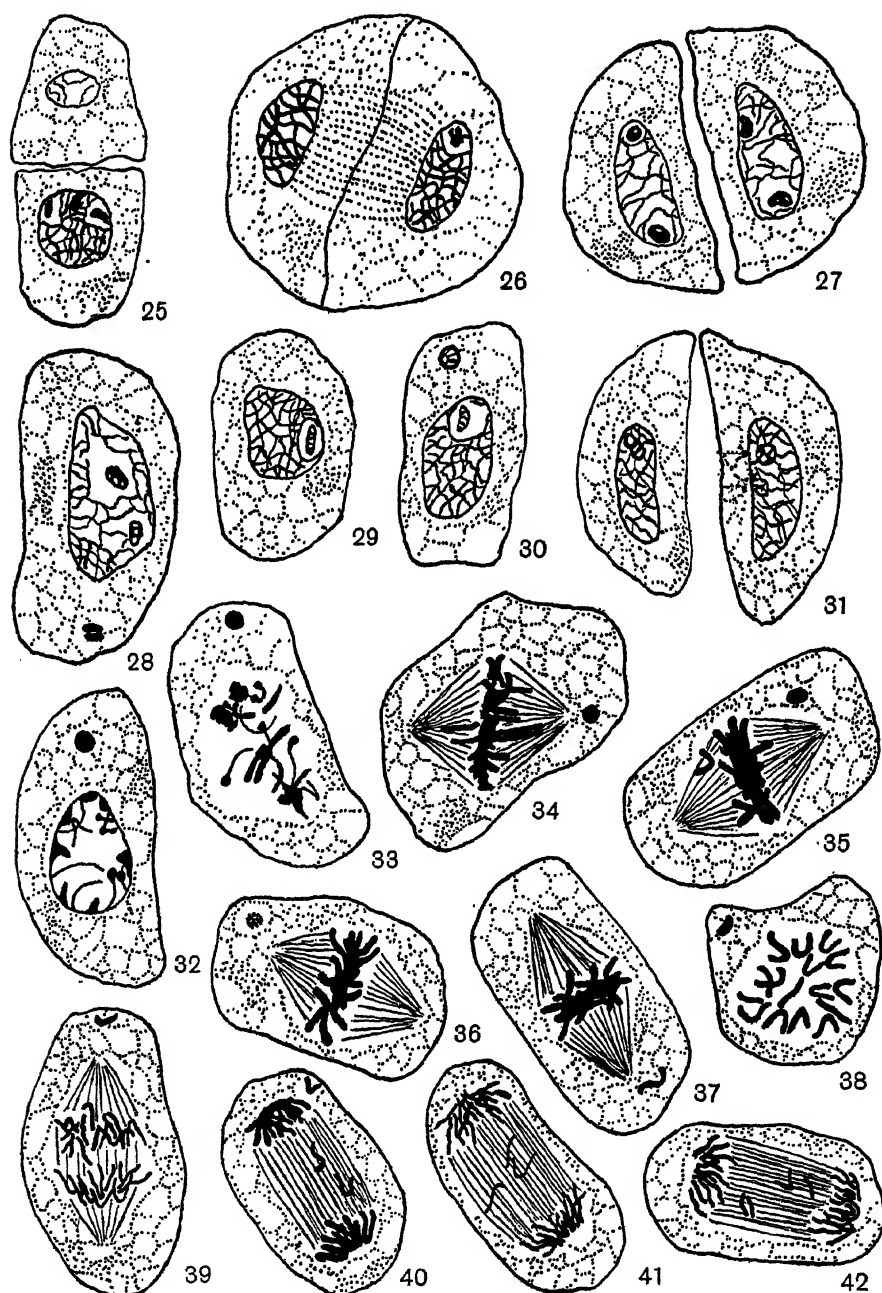
FIGS. 69-77. Microspores.



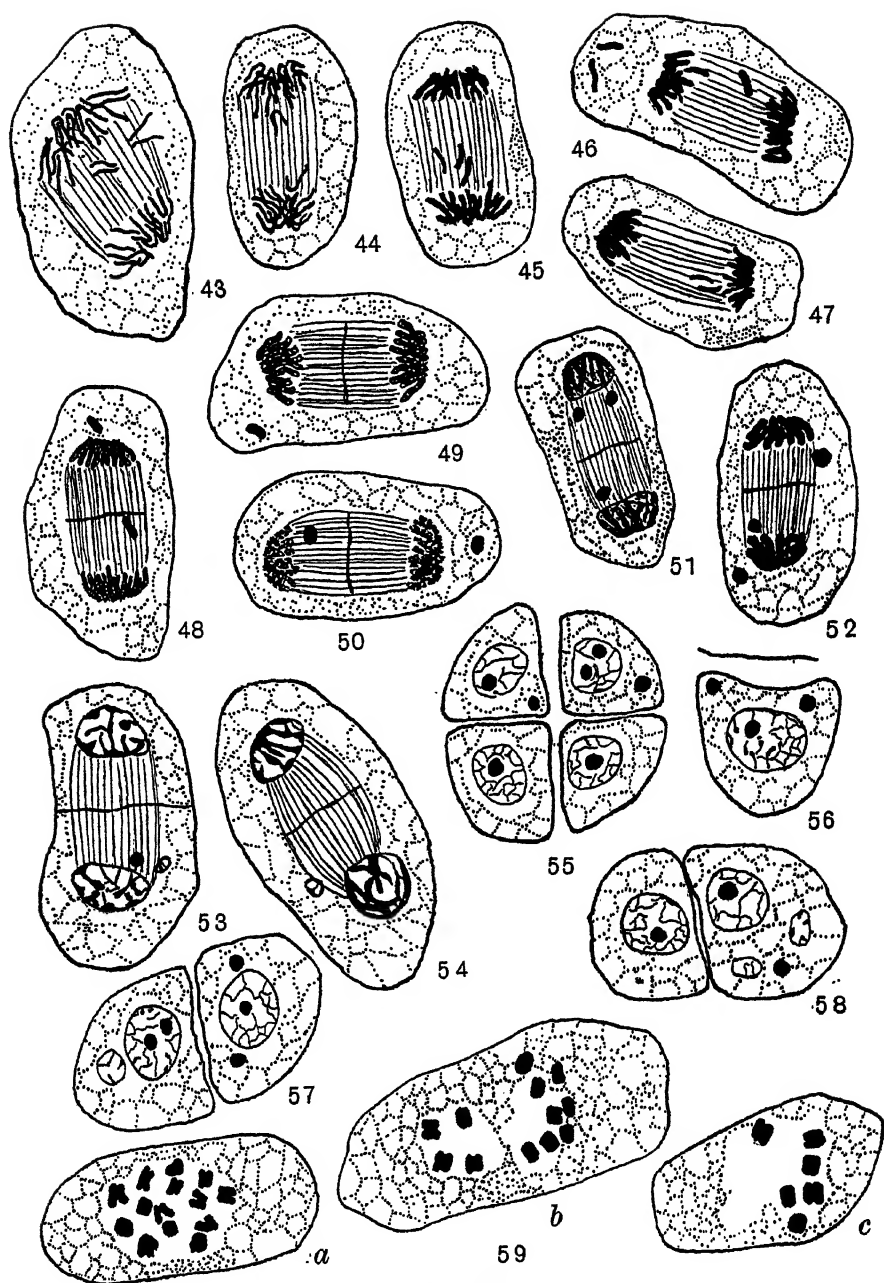
Figs. 1-13.



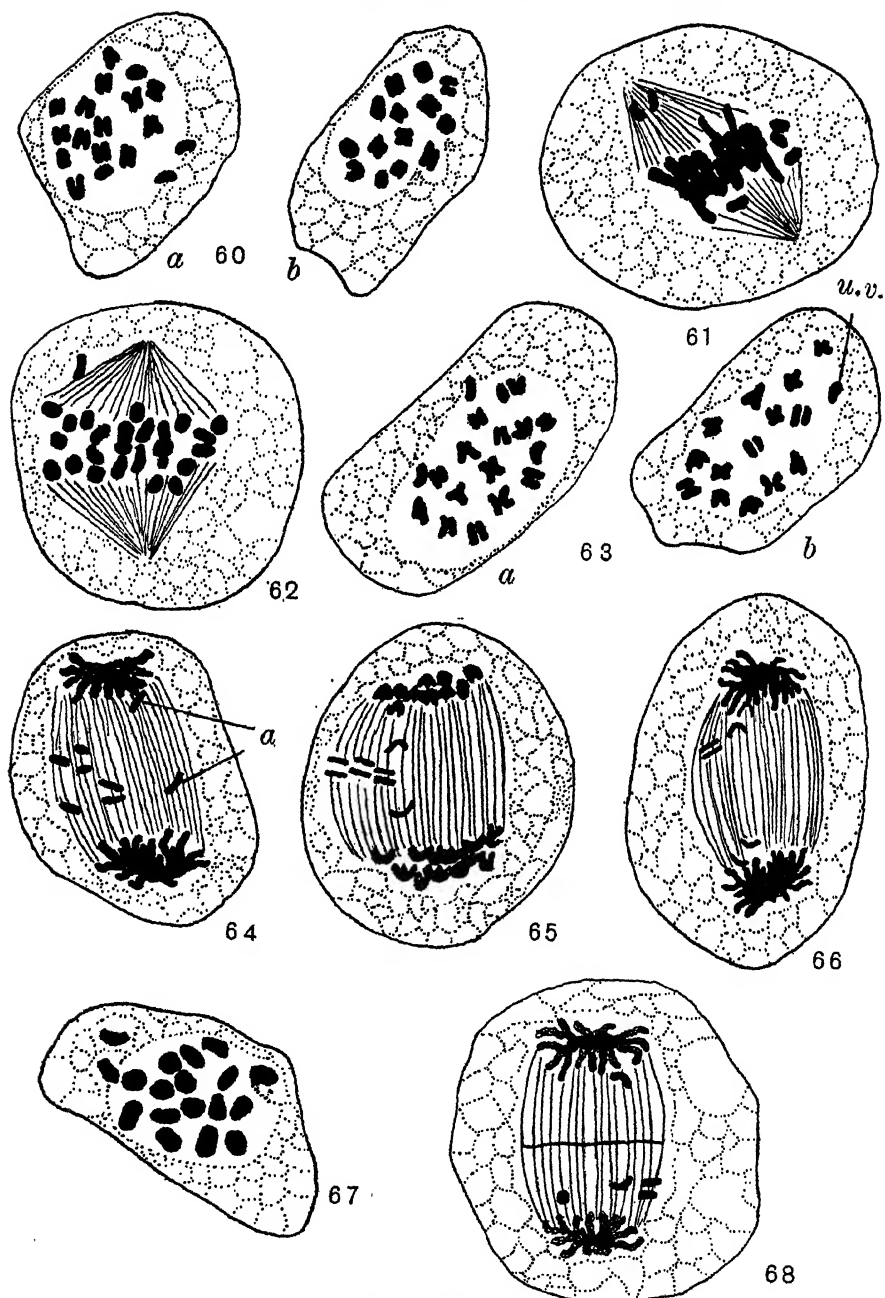
Figs. 14-24.



Figs. 25-42.



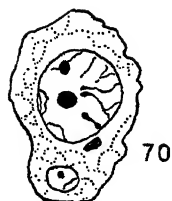
Figs. 43-59 c.



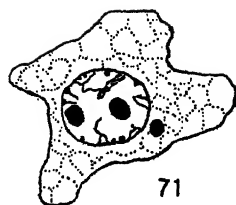
Figs. 60 a-68.



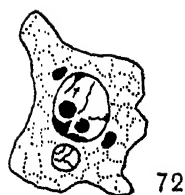
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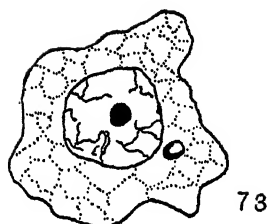
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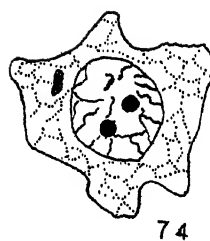
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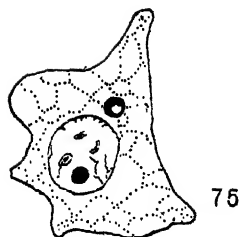
72



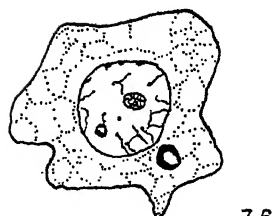
73



74



75



76



77

Figs. 69-77.





# NOTES ON THE GENITALIA OF A CROWING HEN.

By

J. BRONTË GATENBY, M.A. (Dubl.), D. PHIL. (Oxon.), D.Sc. (London)

AND

F. W. ROGERS BRAMBELL, B.A., B.Sc. (Dubl.).

*(Department of Zoology, Trinity College, Dublin.)*

(Four figures in text.)

## INTRODUCTION.

THE hen herein described was a normal bird for the first two years of its life. Whether it ever laid any eggs could not be ascertained, but its owner looked upon it as being a normal hen, until later it began to crow, and to develop a heavy comb and wattles.

When the hen came into our possession it was three years old, and except for its head and for its small spurs, was a normal bird. The head bore a large comb, and the beak and wattles were developed like those of the cock. Its spurs were not much more than buttons.

A hen was introduced into the cage containing the abnormal hen, and the latter immediately became interested; it toyed with a little piece of food, and tried to attract the attention of the hen in the usual manner of roosters. When the unsuspecting hen approached, the abnormal bird did not try to tread, but violently attacked the hen. That is to say the abnormal hen possessed some of the courting attributes of the rooster, but these did not follow out their normal course. The abnormal bird crowed loudly and often.

Birds similar to this are well known, but the present example happens to be a specially interesting case of intersexuality.

We wish to thank<sup>1</sup> Professor Baxter of Trinity College, Dublin, for presenting us with the bird herein described.

When Dr Crew heard that we were studying an intersexual hen, he generously answered any questions and also sent advance copies of the paper of Miss Fell, and of his own interesting paper on sex-reversal.

<sup>1</sup> We must also thank Professor J. P. Hill and Dr J. A. Murray, whose knowledge and advice enabled us to give a correct interpretation of some difficult parts of this work.

The testis in our hen was found by one of us before we knew much about Dr Crew's work, and we were also able to confirm the view of Dr Crew and Miss Fell that the new gonad had originated from peritoneum, before we knew of their work. Nevertheless we owe much, not only to Dr Crew's interpretations of his very complete series, but also to Miss Fell's interesting histological and cytological study.

In two ways our case is unique and especially helpful: the new gonad which is being formed lies both inside and partly outside the old one, and the presence of an enormous quantity of fat does not appear to have been noted by Dr Crew or Miss Fell.

#### PREVIOUS WORK.

The most important contribution to the study of sex-reversal in birds is undoubtedly furnished by Dr Crew of Edinburgh University in the second number of his series of *Studies on Intersexuality*, entitled "Sex-reversal in the fowl." He divides fowls with abnormal sexual characteristics into several classes, the chief of which are: (a) Hermaphrodites which never develop the distinctive characteristics of either sex, but remain intermediate; (b) Otherwise normal females with male plumage; (c) Apparently normal laying hens which on growing old develop more or less completely the male characteristics. The birds belonging to the latter class are generally of heavy laying strains. When two or three years old they cease to lay, the head furnishings rapidly increase in size, and the spurs grow; they attempt to crow, and the plumage may change into the male type. Very rarely such changes may proceed so far as to produce a functional male which, except for a slight difference in shape and carriage, is indistinguishable from a normal cock. Dr Crew further subdivides these cases into (1) those in which the ovary has been more or less completely destroyed by haemorrhage, tumour or atrophy, and (2) those in the gonads of which definite spermatogenic tissue was developed. In the paper referred to he describes at length eight cases, forming a series, belonging to this later subdivision. No. 1 was a Buff Orpington which had been a good layer and a mother of chickens. This bird changed completely and took on all the male characteristics, including the plumage. Two chickens, one a male and the other a female, resulted from mating it with a virginal hen. The post-mortem examination revealed extensive abdominal tuberculosis and a large tumour was found in the situation of the ovary. A testis was present on each side; that on the left being incorporated in the tumour. A thin oviduct was present on the left side and there were paired vasa deferentia. No. 2 was a Rhode

Island Red which retained the henny feathering. A testis was present on the right side and an ovotestis on the left. There were two oviducts and two vasa deferentia. No. 3 was also a Rhode Island Red, with an intermediate plumage. A testis and vas deferens were present on the right side, and an ovotestis, containing some oocytes, and oviduct on the left. No. 4 was a White Leghorn with hen feathering. An oviduct, ovotestis and vas deferens were present on the left side. The four remaining members of the series, three of which were White Leghorns and the fourth a Light Sussex, each had an atrophic ovary with an oviduct on the left side. The spermatatic tissue was represented in different stages, from proliferating sex cords, to old atrophic tubules.

Miss H. B. Fell deals in a separate paper with the histology of the same series of specimens. She shows that fibrous and cystic degeneration of the ovary are followed by the production of the new spermatatic tissue. First the peritoneal epithelium becomes thickened in regions giving rise to a new growth of sex cords. The new sex cords appear to develop rapidly into tubules. In some cases ripe spermatozoa were present in the mature tubules, and spermatogenesis was active. In other cases the mature tubules had atrophied and only spermatogonia were present. In others the young tubules had partly atrophied. A considerable variation was found, even in the individual gonad, in the state of development of the sex cords or tubules. Sometimes new tubules were formed in the cystic follicles, either from the epithelial elements of the follicle or by the penetration of new sex cords.

In most cases Miss Fell records the occurrence of many luteal cells in the testicular tissue, probably having arisen, as in the embryo and young chick, from a fatty degeneration of regions of the sex cords. No. 1 was the only example which had assumed completely the male plumage; no luteal cells were observed in it.

In all, the spermatatic tissue is described as occurring median to the ovarian portion.

The principal literature concerned with the subject is referred to in the bibliographies attached to these two papers.

In our opinion it is an important and remarkable fact that although Dr Crew has examined so many cases of reversed sexuality in the fowl, and so many more have been described less carefully by other observers, all exhibit a change of female towards male characteristics. No case of change in the reverse direction has, we believe, ever been recorded. The significance of this fact will, however, be further considered in the discussion.

## THE RESULTS REVEALED BY DISSECTION OF THE ABNORMAL HEN.

This bird was chloroformed and opened up. To our astonishment we found that its entrails were completely surrounded by masses of fat. One and a half inches overlaid the viscera, and seven ounces were

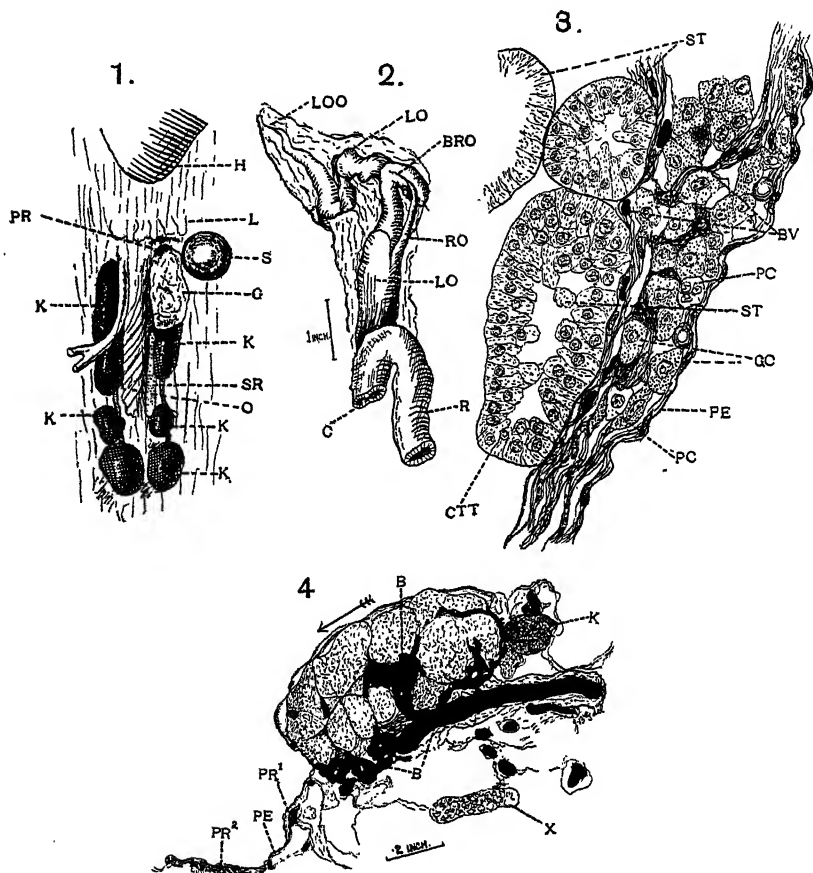


Fig. 1. View of the urino-genitalia of the crowing hen, after removal of intestines and oviduct. *H*, heart; *L*, liver ligaments; *S*, spleen; *PR*, region in front of old gonad (*G*) where new tissue is proliferating; *K*, kidney; *O*, oviducal ligaments; *SR*, left supra-renal.

Fig. 2. Oviducts: *LOO*, oviducal opening; *LO*, left oviduct; *RO*, right oviduct; *R*, rectum; *C*, cloaca; *BRO*, blind end of right oviduct.

Fig. 3. Region of newly proliferated testis. *ST*, spermatic tubules; *BV*, blood vessel; *PC*, pale cells; *GC*, granular cells; *PE*, peritoneal epithelium; *CTT*, connective sheath of spermatic tubules.

Fig. 4. Sagittal longitudinal section through gonad (*G* in fig. 1), showing blood areas (*B*), kidney (*K*), peritoneum (*PE*), new growing areas (*PR*<sup>1</sup> and *PR*<sup>2</sup>), and enigmatic gland at *X*.

removed from the body cavity before the alimentary tract could be cut away. No tumours or tuberculous areas were apparent, and except for the fat the animal appeared to be normal.

A wad with formalin was thrust into the body cavity and next day the dissection was continued. The oviduct is shown in Fig. 2; it was small, but normal, and the lumen (*LOO*) appeared to be open: we could not find any evidence of abnormality, except for the small size of the organ. The oviduct and rectum were removed and the overlying urino-genital organs examined. There was a small gonad on the left side (Fig. 1, *G*), and the surrounding organs (i.e. suprarenal, we found one only, kidneys and blood vessels) seemed fairly normal. No evident tumour or haemorrhage was noticed—fat covered nearly everything, though the muscles and externals of the bird were normal.

With a pair of curved scissors the gonad and its surroundings were carefully removed, cutting well into the retro-peritoneal walls.

This precaution proved important, for as shown in Fig. 4, new growing tissue was found in process of formation outside the area of the old gonad; in this figure the gonad has been drawn in longitudinal sagittal section, the kidney (*K*) at the posterior end, the arrow pointing towards the anterior end. The gonad ends just behind the letters *PR*<sup>1</sup>. The latter letters and also *PR*<sup>2</sup> mark the position of two areas of the peritoneal epithelium beneath which lie peculiar areas of growing tissue.

#### THE RESULTS OF A HISTOLOGICAL EXAMINATION OF THE GONAD AND ITS SURROUNDINGS.

The gonad contained no eggs, though some scar and sclerosed tissue had the appearance of having been formed about old follicles. The main stroma of the gonad was typically that of an effete and sclerosed ovary; we feel that there is little doubt that the main part of the gonad had been an ovary, and probably contained eggs at one period of the bird's life.

In Fig. 4 the gonad is shown to be formed of areas of various kinds of tissue, but there was a good deal of extravasated blood present. No marked phagocytosis was noticeable.

The areas shown in Fig. 4 were marked off partly by connective tissue, but also by variation in the stroma itself. In some places large areas of tubules were noticed of the type shown in Fig. 3. We feel sure that these tubules, which remind one particularly of a tubulo-racemose

gland, are testicular in nature, and do not resemble kidney tubules of any sort. In certain regions of these tubulo-racemose structures are found the smaller round structures which might be ducts. These ducts connect to the tubes which are undoubtedly spermatie, and seem to be forming vasa. During the dissection of the bird we found nothing which might have been a *vas deferens*, or which was in connection with this part of the gonad. In Crew's cases a new vas seemingly must have developed in some unknown manner. The vas-like nature of the tubes in our bird was pointed out to us by Dr J. A. Murray. So far as the pathology of this bird is relevant, we found much haemorrhage, but nothing which the pathologists could certainly identify as an adenoma.

Many of the epithelial downgrowths strongly recalled the normal developing vertebrate testis, a view supported by Dr Murray, and independently expressed by Dr J. P. Hill. In fact our bird seems to be closely similar to one of Dr Crew's White Leghorns with proliferating sex-cords.

In other parts of the ovary the epithelial tubes were smaller, as already mentioned, and these are certainly spermatie tubes. Miss Fell photographs something very like them in her Fig. 17.

*Peculiar New Tissue* was found by one of us in the regions *PR*<sup>1</sup> and *PR*<sup>2</sup> in Fig. 4, just in front of, and outside the old gonad. With Mann's methyl-blue-eosin, the new areas (Fig. 4) took on that beautiful pinkish blue shade which is characteristic of growing tissue: the latter *was* growing and active: it stained like young testicular tissue, but our identification of this tissue as testicular was not complete.

From the general appearance, and from the point of view of the nuclei and the cytoplasm, we think that this new tissue is testicular. No spermatocytes were present, only what might be spermatogonia.

*The origin of new Germ Cells* could be plainly traced in our material. In Fig. 3, the peritoneal epithelium (*PE*) is interrupted and its constituent cells have partly metamorphosed into the large pale cells *PC*, and the granular cells *GC*. These are the forerunners of the testicular cells, shown in Fig. 3, *ST*. We thus support Miss Fell and Crew in their startling claim that the new testicular tissue has directly metamorphosed from peritoneal epithelial cells, a claim which one of us made some years ago for the frog. Our present observations were made independently of the above authors, and were in no way influenced by their work.

As a matter of fact the stages we possess are so early, and their location outside the old gonad is so clear, that we intend to enter into

this matter in another paper, when Miss Fell's work will be noticed more fully. We may say however that it is a great satisfaction to us to find in the work of Crew and Miss Fell such independent and clear confirmation of the view that peritoneal cells do metamorphose into germ cells.

*On the Connection between Gonad and Suprarenal.* In most of our sections the suprarenal has been cut across connected to the old gonad by hypertrophic layers of peritoneal epithelium. Only the upper flakes were present, and until Dr J. P. Hill had examined one of our sections, we were unable to come to a correct conclusion as to the nature of these structures. Both Dr J. P. Hill and Dr J. A. Murray identified these flakes as suprarenal tissue, a view which both of the present writers accept fully. The suprarenal in birds according to Dr Crew (*in literis*) is often connected with the gonad in the adult bird, and always in the chick. We believe that the chromaffin tissue so closely resembles the interstitial tissue of vertebrate testis, and the so-called luteal cells of birds, that histologists investigating bird intersexes, would be well advised to be careful not to misinterpret groups of chromaffin cells for sex gland cells of various types. We ourselves nearly made a mistake in this manner.

In our bird all the epithelium between suprarenal and gonad, and over the suprarenal itself, is in a peculiarly active state, a condition which we cannot explain, but which is symptomatic of the remarkable change which takes place in these birds.

#### DISCUSSION.

Crew, to whose work we owe a great deal of the evidence of intersexuality among the vertebrate animals, has discussed these phenomena in his recent paper on "Sex-reversal in the fowl." In this contribution he describes a number of fowls which display a consistent series illustrating the conversion of an actively functioning hen into an actively functioning cock. The first bird of his series is the most clear-cut instance of natural sex-transformation in vertebrate animals yet recorded. It has been shown that a fowl which previously had been equipped with the sex-organization of the female and had functioned as such, may undergo such transformation as to come to possess the sex-organization of and to function as a male.

In the present paper we have also been able to describe a White Leghorn hen which is beginning to transform into a male, and we are able to confirm many of Crew's results and to corroborate some of his



new interpretations. Crew has broken away from the older views as to the significance of the ovarian tubules which some persons have regarded as mesonephric elements, and has endeavoured to bring his results into line with Goldschmidt's hypothesis of a time mechanism of sex.

The presence of an enormous quantity of fat inside the visceral cavity of our bird has already been noted. It will be agreed by most people that this fat is the outward sign of an anabolic type of metabolism which was dominant at one time in the life of this bird. Immense quantities of fat, glycogen, yolk, etc., which ordinarily went into egg-formation were still being produced after ovarian atrophy, and were deposited in the peritoneal cavity. We have no complete index as to what type of metabolism was dominant when this bird was killed, because fat deposition may have ceased, and the balance may have passed over to a more katabolic or neutral type of metabolism, or at least to one in which vast quantities of fat were no longer being elaborated.

Gonadectomy in birds has been shown to give the following results: castration of the cock produces few changes in the plumage, the spurs are hardly altered, while the castrated bird seldom crows. The capon is not pugnacious, and does not court the hens, but if one of the latter squats down he will mount and go through the characteristic mating reaction. The comb is extremely small, much smaller than that of the hen (Morgan and Goodale).

Pézard found castration inhibited the growth of the comb and wattles, the capacity to crow and the fighting instinct.

Ovariectomy in birds may cause the hen to assume the full plumage of the cock, with spurs, but comb and wattles irregularly developed: such birds do not crow (Goodale).

Pézard concluded that ovariectomy had no influence on the development of the comb, and that the spayed hen was more of a neutral type than male. By transplanting ovarian tissue into a castrated male the growth of spurs, etc., could be inhibited, and the birds feminised.

From these cases it seems likely that the presence of testicular tissue produces the development of the comb and wattles characteristic of the cock; while the presence of ovarian tissue produces the development of the hen feathering, and inhibits the growth of the spurs.

In interpreting the present case on this theory we would assume that hormones capable of causing the development of the female type of plumage and preventing the growth of the spurs, were being produced at least up to the time of the last moult, by the degenerating ovary. At

the same time the growing testicular tissue was producing substances stimulating the growth of the erectile appendages, and possibly causing the bird to crow.

This theory is certainly attractive in its simplicity, and would admirably fit the phenomena were it not for the few cases of bilateral gynandromorphic birds recorded, a good example of which is alluded to by Doncaster. This bird was a Bullfinch completely male feathered on the one side and female feathered on the other side.

Geoffrey Smith, who was the first person to formulate a Mendelian theory of sex which did not involve selective fertilization and reversal of dominance in two sets of individuals of the same species, stated (*Q.J.M.S.* Vol. Lrv, 1910): (1) that in certain species of animals the male is a heterozygote of the composition ( $\delta\eta$ ), while the female is a pure recessive of the composition ( $\eta\eta$ ); (2) that the sexual constitution is not necessarily the same in all species of animals, i.e. the female may be a heterozygote. Bateson and Punnett, in 1908, suggested that the currant moth ♀ was a heterozygote ( $\eta\delta$ ), the male a homozygote ( $\delta\delta$ ) (*Science*, N.S. Vol. xxvii, 1908).

The view we favour, at the present moment at least, is that of most zoologists who have studied the sex question so far as it affects poultry, and enunciated by Punnett in his latest work *Heredity in Poultry*, viz. "The cause in the normal difference between the sexes lies in the hen. She is potentially a bird with cock plumage containing, like the cock, all of the factors necessary for its development." However, as Punnett goes on to explain, there are cases where cocks with henny plumage occur, as for instance the Sebright Bantam. Punnett mentions that he knows of no case recorded in which a normal cock developed the hen type of feathering.

The whole question of intersexuality and sex-reversal and the sex-chromosome hypothesis is interesting. We have no remarks to make except to say that it is certainly peculiar that a determined female should be able naturally to metamorphose into a male. A lifetime of femaleness with female plumage, female genitalia, and supposedly the female chromosome mechanism is over-ridden, and the new tissue formed is testicular. It is hard to understand why the female sex-chromosome mechanism has lost its potency. The female sex in birds seems much less stable than the male. The cells of the peritoneum which metamorphosed into testicular tissue were determined female-cells presumably containing the right female sex-chromosome arrangement. If we cannot depend on the sex-chromosomes what are we to depend upon? At all

events it must be acknowledged that sex does not depend wholly on the sex-chromosomes during all stages of life, and that maleness and femaleness are not stable peculiarities of so-called determined male or determined female cells. If the sex-chromosomes can be over-ridden even after an animal has been established in one sex for years, it seems permissible to believe that the sex-determination apparatus might also during fertilization and ontogeny be over-ridden by hormones from the body of the mother or from parts of the differentiating body of the foetus. Such cases of sex-reversal as have been shown to occur in birds, amphibians and lampreys, open up wide possibilities as to sex-determination in general and permit us to break away from hypotheses of sex-determination which have hitherto denied the possibility of sex-reversal and of sex-determination by any means other than the sex-chromosomes. The sex-chromosome is not omnipotent—so then the door is opened once more to the nutrition-metabolism theorists and to the various opponents of the sex-chromosome theory and its many ramifications.

In conclusion we readily admit the fact of the completeness of the evidence for the chromosome theory, and for the theory of sex-determination by means of a sex-chromosome mechanism. However the body of evidence concerning sex-reversal has been growing and demands explanation. We are driven by it to the theory that sex is reversible throughout life in certain cases and under favourable conditions.

It cannot be denied that these two theories, while not being contradictory, detract from each other, but the broader principle which underlies both has so far evaded our grasp.

#### SUMMARY.

1. A white Leghorn fowl is described which was completely henney except for its head, which resembled that of a cock bird, possessing a large comb and wattles.

2. The bird courted the hens up to a certain point, after which it savagely attacked them: it crowed loudly and often.

3. On opening the body cavity large masses of fat were found covering the viscera. One and a half inches covered the intestines, and seven ounces were removed before the genitalia could be exposed.

4. The oviduct was normal, though small.

5. On the left side was a flat gonad, not testiculiform in shape. A section of this gonad revealed that it was an ovary full of scar tissue:

numerous tubules were present, and almost filled the gonad. These were pronounced by various authorities to be testicular.

6. In front of the ovary, and completely outside it, were many areas of newly proliferating tissue which were possibly testicular. Normal spermatid tubes with spermatogonia were not present.

7. The new spermatid cells arose by transition of peritoneal epithelial cells into germ cells.

#### POSTSCRIPT.

Since this paper was written we have received a contribution from Hartman and Hamilton (*Jour. Exp. Zoology*, Vol. xxxvi, No. 2, Aug. 1922), in which is described a hermaphrodite Rhode Island Red which possessed a testis and an ovotestis. I believe that the sensitive area on the right side of the bird's peritoneum, where the atrophied gonad would have been situated, is responsible for a number of these hermaphrodites. The sensitive peritoneum instead of remaining inactive grows a new gonad which is probably always a testis in the cases of hermaphrodite hens. J. B. G.

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# STRUCTURAL MOSAICS AND INHERITANCE OF VARIEGATION IN *BARBAREA VULGARIS*

By IRMA ANDERSSON.

*The John Innes Horticultural Institution.*

(With 6 Plates.)

A VARIEGATED form of *Barbarea vulgaris* is well known, and is often to be seen self-sown in gardens into which it has been introduced. Mr E. A. Bowles kindly gave several of these plants to the Institution, which were the origin of these experiments.

The variegation consists in the presence of yellowish-white areas in the leaves, and sometimes in the stems. These patches are of various sizes, larger and smaller. The distribution of the variegation differs from that characteristic of sectorial chimaeras and evidently belongs to a different class. The distinction is not easy to express. It consists not so much in the fact that the variegation is of the nature of an intimate mixture, inasmuch as some obviously sectorial mosaics show an even more intimate mixture of the two types of tissue (e.g. *Primula sinensis*, Carnation flake). The distinction is rather one of geometrical arrangement. In the sectorial mosaics the lines of division are sharp and the contrasting areas are arranged in wedges, each wedge having obviously arisen by division of a common original cell or group of cells. In the *Barbarea* the patches are commonly of widely irregular shape, and the appearance suggests that something has spread through the tissue, affecting the cells of which it is composed. Nevertheless, sectorial divisions in regard to variegation have frequently been seen in the plants, but the segments to be distinguished are not respectively *green* and *yellowish-white*, but green on the one hand, and the variegated mixture on the other. In general the yellow areas run out to the margins, though occasionally they may be bordered by green tissue. On the contrary the yellow areas almost always surround patches of green and apparently normal tissue, which may be of considerable size or merely green specks (Fig. 1).

A peculiar feature, not hitherto recorded as far as I know, appears in the frequent occurrence of white areas in the yellow *petals*.

As stated above, the variegated form commonly maintains itself in gardens from seed. This behaviour also distinguishes it at once from the mass of variegated plants, whether sectorial or periclinal mosaics. But since, though the geometrical arrangement may be peculiar, the vegetative cells are nevertheless greens and not-greens, simple expectation might lead us to suppose that there would be gametic cells corresponding to each of these two classes. Experiment shows however that, on the whole, the variegated plants breed true in variegation, and that therefore their germ cells transmit the combination, not the components; which is contrary to the expectation suggested by ordinary mosaics. An analogous case, that of a variegated *Tropaeolum*, had been under investigation at the Institution for some years when these *Barbareas* were seen in Mr Bowles's garden. The variegated *Tropaeolum* will form the subject of another paper. It breeds true, can be transmitted either by egg-cells or pollen in crosses with pure greens, and then behaves as an ordinary recessive. No green segments have ever been seen in it.

Dahlgren began experiments with *Barbarea* at about the time ours were begun, and he published in 1921<sup>1</sup>. His work, and that about to be related, show that in spite of the resemblance between the two cases, the *Barbarea* presents complications not met with in *Tropaeolum*, notably such differences in numerical composition of  $F_2$  as to create a strong presumption that both 3:1 and 15:1 may occur as ground-numbers, and moreover that true green or variegated sectors may be formed with special genetic properties.

The experiments here described relate to crosses between *vulgaris variegata*  $\times$  *vulg.* type. They were intended to test the inheritance of variegation. A remarkable feature however, which must be recorded, is the production of *sectorial mosaics in structural characters*, apart from colour, seen in the plants resulting from crosses between the variegated and the green types in the one species, *B. vulgaris*. The sectors of such mosaics in some instances have the characters of the less common  $F_2$  forms.

In addition to these experiments, many species-crosses were made, introducing the species *stricta*, *praecox* and *intermedia*. The results were highly complex and led to no conclusion sufficiently definite to be related here. Though thousands of descendants were grown from such crosses, no forms appeared resembling the forms of the polymorphic  $F_2$  and  $F_3$  described below.

<sup>1</sup> Dahlgren, K. V. O., "Vererbungsversuche mit einer buntblättrigen *Barbarea vulgaris*," *Hereditas*, Vol. II. 1921.

Eleven  $F_1$  plants were raised by Mr Bateson from variegated *Barbarea vulgaris* as ♀ × the normal green as ♂. These  $F_1$  plants were wholly green. Four were kept in an isolating house: two were self-fertilised: and the spikes of the other two were brushed together. The seed from the latter pair may therefore have resulted either from self-fertilisation or from *inter se* mating. The  $F_2$  families are set out in Table I, in all 131 green, 24 variegated. Of the latter, though all showed variegation, 6 were obviously sectorial mosaics of wholly green parts combined with fully variegated parts. Apart from these sectorials the amount of variegation differed greatly, most of those reckoned as variegated resembling the pure variegated type, but some showing occasional patches of variegation.

From these  $F_2$  plants families were raised by various matings as shown in Tables II and III. In Table II the results from uniform<sup>1</sup> plants are given, those from sectorials being shown in Table III.

Owing to the exceptionally dry summer in 1921, and insect pests, as well as an attack of *Albugo* (*Cystopus*) in 1922, most of the  $F_3$  plants were lost, but a segregation of 15:1 or 3:1 from the selfed  $\frac{1}{19}$  and  $\frac{2}{19}$  families seemed likely. Only three families of selfed  $\frac{3}{19}$  plants contained enough individuals when in flower to be considered here. Of these it appears that the variegated plants  $\frac{3^{23}}{19}$  and  $\frac{3^{51}}{19}$  bred true for variegation. The very much variegated  $\frac{3^{47}}{19}$ , however, selfed gave 65 variegateds and 5 greens. This mother-plant  $\frac{3^{47}}{19}$  showed both the fine and the coarse mixture of green and variegated parts, with a maximum amount of white, characteristic of a strain  $\frac{150}{21}$  which breeds quite true for this amount of variegation. The variegated plants of family  $\frac{47}{21}$ , however, were not equally variegated. Some showed white patches already in the first leaves, or even in the cotyledons. Others were entirely green up to a few leaves under the inflorescence, where after careful scrutiny eventually a white patch was discernible. In the 5 green plants mentioned no sign of variegation whatever was seen. The normal inheritance of the

<sup>1</sup> As opposed to the definite sectorials.



# 188 *Inheritance of variegation in Barbarea vulgaris*

variegated  $\frac{47^{163}}{21}$  is seen in the table.  $\frac{47^{800}}{21}$ , a similar variegated plant of the same family, might have been of similar nature, had it not given one variegated besides the green, when crossed with the true-breeding green  $\frac{16}{21}$ . Of the 5 green descendants from the variegated  $\frac{3^{47}}{19}$ , mentioned above (in  $\frac{47}{21}$ ), one gave a variegated progeny, and the inheritance in another  $\frac{47^{500}}{21}$  was, as appears from the table, in spite of the small numbers, evidently anomalous.

## CHIMAERAS.

These were of three kinds:—

*Class I.* Plants having two sharply distinguished parts (stems, leaves, etc.), one green, the other variegated.

*Class II.* Plants like the foregoing, but exhibiting structural distinction between the green and variegated parts respectively. In these the division was perfectly sharp.

*Class III.* Plants wholly green, which showed two parts distinct structurally.

In all these chimaeras the plants consisted of what may be called two distinct halves, in the sense, not that the respective parts were equal in size, but that there was no irregularity or overlapping.

## *Class I.*

TABLE I

$F_1$			$F_2$			
No.	Description	How fertilised	No.	Description	Green	Sectorially gr. vargd.
— 6 <sup>1</sup>	Green	Selfed	$\frac{2}{19}$	25	8	2
— 6 <sup>4</sup>	„	„	$\frac{1}{19}$	29	5	1
— 6 <sup>3</sup>	„	× 6 <sup>2</sup> or selfed	$\frac{3}{19}$	49*	5	3
— 6 <sup>2</sup>	„	× 6 <sup>3</sup> or selfed	$\frac{4}{19}$	28†	—	—

\* Includes one mosaic in structural characters.

† Includes two mosaics in structural characters.

TABLE II

Parents			Resulting family		
Reg. No. of Mother Plant	Description	How fertilised	Reg. No.	Green	Variegated
$\frac{3^{23}}{19}$	V. *	Selfed	$\frac{43}{21}$	—	23
$\frac{3^{51}}{19}$	" *	"	$\frac{48}{21}$	—	60
$\frac{3^{17}}{19}$	" *	"	$\frac{47}{21}$	5	65
$\frac{47^{163}}{21}$	" *	$\times \frac{150^*}{21}$ true-breeding + recip.	$\frac{110-111}{22}$	—	31*
$\frac{23^{166}}{21}$	" true-breeding	$\times \frac{47^{163}}{21}$	$\frac{88}{22}$	—	4*
$\frac{47^{800}}{21}$	" *	Selfed	$\frac{100}{22}$	—	25*
$\frac{47^{800}}{21}$	" *	$\times \frac{150}{21}$ true-breeding + recip.	$\frac{104}{22}$	—	1*
$\frac{47^{800}}{21}$	" *	$\times \frac{16}{21}$ gr. true- breeding	$\frac{103}{22}$	8	1
$\frac{47^{800}}{21}$	" *	$\times \frac{47^{700}}{21}$ gr.	$\frac{101}{22}$	—	13
$\frac{47^{700}}{21}$	Gr.	Selfed	$\frac{97}{22}$	—	3*
$\frac{47^{800}}{21}$	"	"	$\frac{106}{22}$	22	—
$\frac{47^{800}}{21}$	"	$\times \frac{150^*}{21}$ true-breeding + recip.	$\frac{109}{22}$	—	11
$\frac{150}{21}$	V. *	$\times \frac{47^{800}}{21}$ gr.	$\frac{112}{22}$	—	30
$\frac{47^{800}}{21}$	Gr.	$\times \frac{16}{21}$ gr., true- breeding	$\frac{108}{22}$	80	—
$\frac{23^{166}}{21}$	V. true-breeding	$\times \frac{47^{800}}{21}$ gr.	$\frac{89}{22}$	6	40

V. = variegated.

Gr. = green.

\* = plants showing much variegation, manifested already in the young rosettes.

The individual  $\frac{2^3}{19}$  had most of its shoots green, but two adjacent shoots were much variegated throughout. Another plant ( $\frac{3^1}{19}$ ) was half variegated and half green. The green shoots were slightly taller but otherwise the two parts differed in no respect.  $\frac{3^{16}}{19}$  further was green except for one much variegated shoot. As appears from the table, both the variegated and the green part of these plants seem to breed true as regards this distinction.

## 190 *Inheritance of variegation in Barbarea vulgaris*

A similar plant  $\frac{3^{37}}{19}$ , however, gave from the variegated part 60 plants  $\left(\frac{19}{21}\right)$  of which 57 had green rosettes and 3 *variegated*. All the 57 green had entirely green shoots. One of the *variegated* rosettes sent up solely *green* shoots  $\left(\frac{19^{104}}{21}\right)$ ; another *variegated*  $\left(\frac{19^{105}}{21}\right)$  had all shoots *green* except for one, which was clearly *variegated*; and further the third *variegated* rosette had all shoots *variegated*. The variegated part of this sectorial  $\frac{3^{37}}{19}$  may thus be considered as a counterpart to the variegated  $\frac{3^{47}}{19}$ , which gave rise to the greens in  $\frac{47}{21}$  (above). The green part of the same sectorial  $\frac{3^{37}}{19}$  gave rise to 49 greens and 2 variegateds, all with green rosettes  $\left(\frac{20}{21}\right)$ .

The variegated shoot of  $\frac{19^{105}}{21}$  (above) again gave rise to 11 *green* plants. The green part of the same plant, giving only greens when selfed, gave, used as ♀, when crossed with a true-breeding variegated, a progeny of 7 green and 32 variegated plants in the first year. Of the 32 variegated, 28 survived and had variegated shoots; and the 29th, which the previous year had had a green rosette, had now variegated shoots. The variegated part of this sectorial  $\frac{19^{105}}{21}$ , used as ♂, when crossed with the same true-breeding variegated, gave 3 early variegated plants.

The individual  $\frac{19^{104}}{21}$  in the same family, which had the rosette variegated but all the shoots green (see above), gave rise to 7 greens and 3 variegateds, when in the big rosette stage; but all sent up entirely green shoots the next year, the variegateds thus repeating the phenomenon of the parent.

It ought to be mentioned that all the recorded plants were grown as biennials (usually not flowering in their first summer), and developed huge rosettes and subsequently a large amount of shoots. All were quite healthy.

### *Class II.*

The two halves of a further sectorial chimaera,  $\frac{1^{12}}{19}$  (Fig. 3), differed in several respects. Half of this plant (part I) had much variegated

shoots. Even the petals had white areas, sharply divided from the yellow ground (Fig. 2). The leaves were of large size, and of the most common *vulgaris* shape with a broad end-lobe and short petiole. Anthocyanin only at lowest part of stems. Shoots of part I were taller and in all respects more robust, and with details of larger size than part II, which latter part was characterised by dark-green and more slender shoots. Anthocyanin distributed in the neighbourhood of the inflorescences, but absent below. Leaves long-stalked, with narrow pointed end-lobe. The variegated part I was sterile, and part II gave rise only to 5 green plants, all with the leaf-shape of part I. The habit of part II is, so far as our experience goes, rare, and occurred only very few times in our experiments. When it appeared, it was sterile.

The green and variegated halves of  $\frac{2^{14}}{19}$  were also different in respect to leaf-form, size, etc. The green part (part II), two shoots, had smaller leaves with peculiarly cut edge, rather pointed end-lobe, clasped along the mid-rib, dark, very leathery, and with smooth surface. The variegated shoots (part I) were characterised by bigger leaves of ordinary texture and by a larger and more rounded end-lobe. The green part (II) gave rise, when selfed, to family  $\frac{6}{21}$ , containing 8 greens (Nos.  $\frac{6^2, 5, 11, 4, 12, 13, 101, \text{and } 9}{21}$ ) and 4 variegateds Nos.  $\left(\frac{6^3, 6, 7, 8}{21}\right)$ . Of these Nos.  $\frac{6^2, 3 \text{ and } 5}{21}$  (see Fig. 5) had the same leaf-form as the mother-sector (II). No.  $\frac{6^6}{21}$  (see Fig. 6) resembled part I, and the rest were intermediate. A new combination in respect to variegation and morphological character has thus occurred. The variegated part I gave rise to family  $\frac{8}{21}$ , with one *green* and one *variegated* plant, both like part I. Part I  $\times$  part II gave one *variegated* with leaf-form as part I, and part I  $\times$  a true-breeding green gave again one *variegated* and one *green*, both like part I. The same part used as  $\delta$  on another true-breeding green gave 39 greens. Thus not even the green of a normally true-breeding green plant dominates over variegation when part I was used as mother  $\left(\frac{10}{21}\right)$ . When the other green was used however we had the complete dominance  $\left(\frac{115-117}{21}\right)$ .

The variegated plant  $\frac{6^3}{21}$ , descended from the green part (II), gave

## 192 *Inheritance of variegation in Barbarea vulgaris*

2 variegateds and 2 greens. A sister plant,  $\frac{6^5}{21}$ , green, gave one variegated and 23 greens;  $\frac{6^4}{21}$ , green, gave 2 greens. Here too the leaf-form similar to part II is heterozygote. The descendants of the intermediate  $\frac{6^4}{21}$  were like part II.  $\frac{6^3}{21}$  variegated (above)  $\times$   $\frac{8^1}{21}$  green (above) gave only one green plant having the leaf-type of part I. The same  $\times$   $\frac{9^1}{21}$  (above) gave one green with leaves similar to part I, and  $\frac{8^1}{21} \times \frac{9^1}{21}$  gave one green individual with intermediate leaf-characters.

### Class III.

The sectorial  $\frac{3^{42}}{19}$  was entirely green, the two parts however being very different in other respects (see Fig. 4). Part I was peculiar in its emerald green colour. The end-lobes as well as the side-lobes of the leaves were big, broad, and with wavy edge. Part II was taller than part I, leaves dark green, leathery, with a smoother surface than part I, and with narrow more pointed lobes. (See also Fig. 7, the two rows of leaves, the upper part corresponding to part II, the under corresponding to part I. The inheritance of leaf colour is seen in Table III.)

TABLE III

Parents			Resulting family				
Reg. No. of Mother Plant	Description	How fertilised	Reg. No. of Family	Rosettes		Shoots	
				Green	Vargd	Green	Vargd
$\frac{2^3}{19}$	V. part	Selfed	$\frac{4}{21}$	3	—	—	3
$\frac{2^3}{19}$	Gr. "	"	$\frac{5}{21}$	4	—	Dead	Dead
$\frac{3^1}{19}$	V. "	"	$\frac{14}{21}$	—	19	—	19
$\frac{3^1}{19}$	Gr. part self-sterile	—	—	—	—	—	—
$\frac{1^{26}}{19}$	V.	$\times \frac{3^1}{19}$ gr. part	$\frac{32}{21}$	33	—	33	—
$\frac{3^{16}}{19}$	V. part	Selfed	$\frac{18}{21}$	—	75	—	72 surviving
$\frac{3^{16}}{19}$	Gr. "	"	$\frac{16}{21}$	199	—	148 surviving	—
$\frac{3^{16}}{19}$	" "	$\times \frac{1^{26}}{19}$ v.	$\frac{17}{21}$	36	—	30 surviving	—
$\frac{18^{71}}{21}$	V.	$\times \frac{17}{28}$ gr.	$\frac{62}{22}$	7	5	7	5

V. = variegated.

Gr. = green.

TABLE III (continued)

Parents			Resulting Family				
Reg. No. of Mother Plant	Description	How fertilised	Reg. No. of Family	Rosettes		Shoots	
				Green	Vargd	Green	Vargd
$\frac{3^{37}}{19}$	V. part	Selfed	$\frac{19}{21}$	57	3	58	2*
$\frac{3^{37}}{19}$	Gr. "	"	$\frac{20}{21}$	51	—	49	2
$\frac{19^{105}}{21}$	" "	"	$\frac{67}{22}$	9	—	9	—
$\frac{19^{105}}{21}$	Gr. part	× true-breeding var.	$\frac{71}{22}$	7	32	6	29
$\frac{19^{105}}{21}$	V. "	Selfed	$\frac{68}{22}$	11	—	11	—
True-breeding var.	—	× $\frac{19^{105}}{21}$ v. part	$\frac{65}{22}$	—	3	—	3
$\frac{19^{104}}{21}$	Rosette v., shoots gr.	Selfed	$\frac{70}{22}$	7	3	10	—
$\frac{1^{12}}{19}$	V. part I	Sterile	—	—	—	—	—
$\frac{1^{12}}{19}$	Gr. part II	Selfed	$\frac{27}{21}$	5	—	5	—
$\frac{2^{14}}{19}$	" "	"	$\frac{6}{21}$	8	4	8	4
$\frac{2^{14}}{19}$	V. part I	"	$\frac{8}{21}$	1	1	1	1
$\frac{2^{14}}{19}$	" "	× part II	$\frac{9}{21}$	—	1	—	1
$\frac{2^{14}}{19}$	" "	× <i>vulgar</i> I gr. true-breeding	$\frac{10}{21}$	1	1	1	1
<i>Vulgar</i> I	Gr. true-breeding	× $\frac{2^{14}}{19}$ part I	$\frac{115-117}{21}$	39	—	39	—
$\frac{6^3}{21}$	V.	Selfed	$\frac{52}{22}$	22	2	2	2
$\frac{6^5}{21}$	Gr.	"	$\frac{51}{22}$	23	1	23	1
$\frac{6^4}{21}$	"	"	$\frac{50}{22}$	2	—	2	—
$\frac{6^3}{21}$	V.	× $\frac{8^1}{21}$ gr.	$\frac{53}{22}$	1	—	1	—
$\frac{6^3}{21}$	"	× $\frac{9}{21}$ v.	$\frac{54}{22}$	1	—	1	—
$\frac{8^1}{21}$	Gr.	× $\frac{9^1}{21}$ v.	$\frac{55}{22}$	1	—	1	—
$\frac{3^{42}}{19}$	Gr. part I	Selfed	$\frac{22}{21}$	146	28	146	28
$\frac{3^{42}}{19}$	" part II	"	$\frac{23}{21}$	121	12	121	12
$\frac{3^{42}}{19}$	" part I	× part II	$\frac{24}{21}$	15	1	15	1

V. = variegated.

Gr. = green.

\* Including one sectorial.

## 194 *Inheritance of variegation in Barbarea vulgaris*

The peculiar emerald green of part I was met with in some of the progeny of part I, but the complexity of the various leaf-characters made a strict classification impossible. The characters making up the habitus of the two parts were probably combined anew among the descendants. Only the most extreme form, namely a combination resembling one or the other of the two mother plant parts, was easily recognised, and stands in the following description under the term "extreme 22 type" (corresponding to part I), and "extreme 23 type" (corresponding to part II of plant  $\frac{3^{42}}{19}$ ), see Fig. 7.

Family  $\frac{22}{21}$  from part I contained 15 "extreme 22 type," and 37 "extreme 23 type." The rest were so complex as not to be described or recorded, except as more or less like one type in one or other respect. Part II gave rise to family  $\frac{23}{21}$  with 117 plants of "extreme 23 type," 4 or 6 more or less "22 type," and two peculiar plants with small leaves resembling  $\frac{6^2}{21}$  (Fig. 5).

The complexity of the leaf-characters was further shown by crossing part I and part II. The result was 5 plants of more or less "23 type," 3 or 4 plants of more or less "22 type," and 8 or 9 intermediates. Several extreme and intermediate individuals in both families were worked upon, the numbers however being too obscure for further analysis.

### CONCLUSIONS.

Although the numbers are small, some features in the inheritance are clearly demonstrated. The facts are:—

1. That variegateds in certain families give off green plants. That these greens subsequently behave either as true variegateds or, when selfed, give greens only; and, when crossed with some true-breeding variegateds, give variegateds and greens, and crossed either way with a homozygote green give rise to greens only.

2. Further, that in the sectorial chimaeras, in some cases, the two parts breed true, respectively, for greenness and variegation: in other cases, do not breed true, not even the variegated part; the inheritance being obscure. (Obs. in some sectorials the green part is sometimes smaller than the variegated part.)



Fig. 1.







Fig. 2.





Part I.

Fig. 3.

Part II.





Part I.

Part II.

Fig. 4.





Fig. 5.







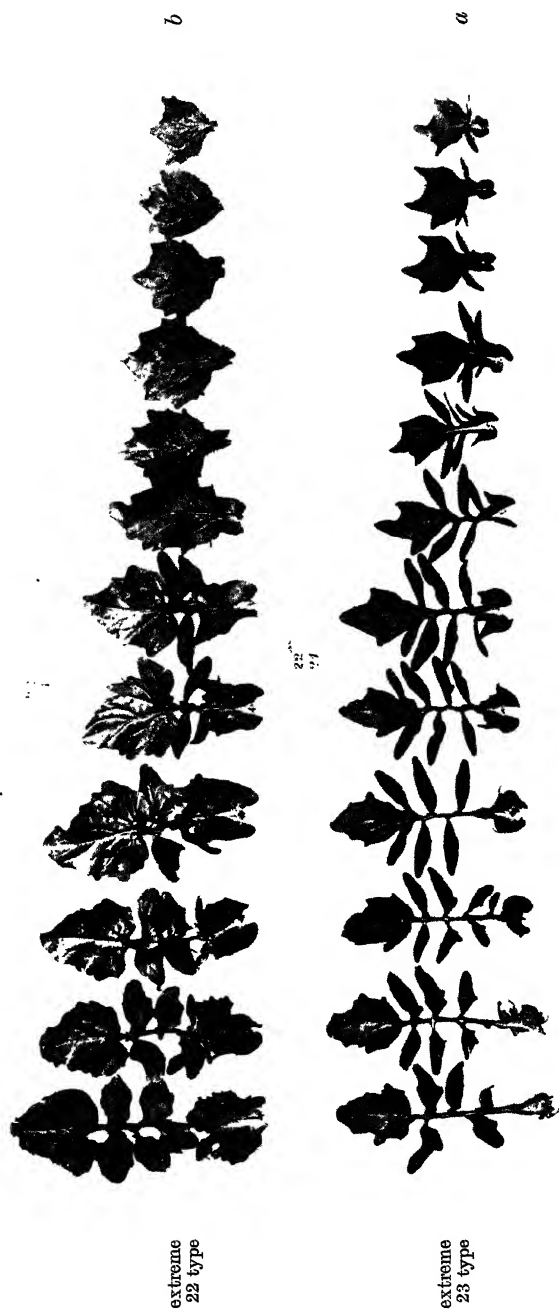


Fig. 7.



3. The clear development of green shoots from wholly variegated rosettes, and these subsequently giving rise to green and variegated rosettes, which again produced solely green shoots.

4. The frequent occurrence of sectorials for various characters, not only variegation. The independence of the two phenomena, and of the occurrence of sterility in some cases, either in the green or in the variegated sector.

5. The white-blotching or white stripes of the petals, probably correlated with a high amount of variegation of stems and leaves.

6. In general, the variegation is recessive in heterozygous combination with green. Gametic segregation in these respects occurs. Families are thus produced in general conformity with Mendelian principles. Nevertheless departures from this simpler plan are not infrequent, which must be attributed to segregation occurring in somatic tissues.

### EXPLANATION OF PLATES III—VIII.

Fig. 1. *Barbarea vulgaris*, variegated. The usual appearance in rosette-stage.

Fig. 2. Flowers showing the pale stripes often occurring on much variegated plants.

Fig. 3. Sectorial  $\frac{1^{12}}{19}$ . See Table III and p. 190. Two parts of one plant. Part I, variegated. Part II, wholly green. Observe also the structural distinctions.

Fig. 4. Sectorial  $\frac{3^{42}}{19}$ . Two parts of one plant, both green but structurally distinct. See Table III and p. 192.

Fig. 5. Green rosette, offspring of green part of sectorial  $\frac{2^{14}}{19}$  (Table III and p. 191); in structure like maternal part.

Fig. 6. Variegated rosette, offspring of variegated part of the same sectorial,  $\frac{2^{14}}{19}$ .

Fig. 7. Leaves from two plants, offspring of the sectorial figured in Fig. 4. The *a* row is from a plant offspring of Part I, the *b* row from a plant offspring of Part II. See p. 194.



## SEED PROGENY OF A POTATO WITH FAINTLY COLOURED TUBERS<sup>1</sup>.

BY JAMES P. KELLY,  
*State College, Pennsylvania, U.S.A.*

DURING parts of the past three summers I have been collaborating with the pathologists, Drs C. R. Orton and F. Weiss, in the attempt to breed a variety of potato, suitable for Pennsylvania conditions, that would resist the wart disease. The most generally prized potatoes are the various "Rurals" which unfortunately are all susceptible to the wart organism. My part in the programme has been the producing of seedling plants through the crossing of "Rurals" with resistant or immune sorts, of which the "Red McCormick" potato is one. The present small contribution on tuber coloration in the "Red McCormick" is a by-product of our project.

The variety "Red McCormick" has really very little red in its tubers. Its tubers are essentially cream with light flushes of carmine in and about the eyes. Guarded selfings of "Red McCormick" plants gave a total of nineteen seedling offspring. To those familiar with horticultural plants that usually are reproduced vegetatively it is not surprising to find seed progeny non-uniform. This proved to be the case with the "Red McCormick" plants that were self-pollinated. Eight of the nineteen offspring were "Red McCormick" in appearance and five were without any trace of anthocyan, that is, were cream. The other six had uniformly distributed carmine and it was surprising to find two of these rather intensely coloured. (See Table I.)

The "Rural" potatoes have cream-coloured tubers without any anthocyan in the skin. The flowers of the "Rurals" seem to be without viable pollen and so we have had no progeny from the selfings of these. Six guarded crossings of "Rurals" with other white-tubered varieties ("White McCormick" and "Irish Cobbler") gave rise to 53 seedling descendants, all with the colourless or cream tubers. We may therefore presume that plants with cream-coloured tubers breed true for this characteristic.

Ten hybridizations (all but two carefully guarded) of "Red McCormick" and cream-tubered varieties of the "Rural" group of potatoes gave a total of 205 seedling plants. This  $F_1$  group was a mixture. Slightly

<sup>1</sup> Contribution No. 44 from the Botany Dept. of the Pennsylvania State College.

## 198 *Seed Progeny of a Potato with faintly coloured tubers*

more than half the plants had coloured tubers and a little less than half possessed cream tubers. Dr R. N. Salaman has published data in the first volume of the *Journal of Genetics* on potato coloration and he refers to a disproportionate mortality of creams as a disturbing element in his results. Possibly the same is true of our material so that our ratio of 107 coloured to 98 cream is really an approach to a 1:1 ratio, a situation very familiar to Mendelian students in back-crosses.

Confining our attention to the 107 coloured members of this  $F_1$  group it appears from Table I that there were three main colour types (designated by the Roman numerals I, II, and III):

*Group I*, which consisted of 31 plants, resembled the "Red McCormick" parent in the slight intensity of colour and in the restricted distribution of the colour.

*Group III*, of almost the same number of individuals as Group I, had the pigmentation also light but evenly suffused over the tuber.

*Group II*, with many more individuals than either Group I or III, had the colour uniformly distributed but deep in shade.

The "Rural" parents possessed a blueing gene and its interplay among the other genes caused each of the three main colour types to present a reddish and a violet edition of itself, in curious numerical relationships. These reddish and violet sub-types may be disregarded in our attempt to seek a possible interpretation for the main features of our data.

A few cases were encountered in which the coloration was difficult to classify; for example, three or four listed as "Red McCormick" had an exceedingly faint flush of coloration over the whole tuber but with rather marked eye pigmentation.

Considering the selfed progeny of "Red McCormick" it is obvious in the first place from the ratio of 14 coloured to 5 colourless that this variety is heterozygous for some simple basic colour factor that we may call B. Further, the fact that the majority of the coloured ones are light while the intensely coloured plants are relatively few, may point to a dominant diluting factor (that we may label D), also heterozygous. The possibility that the light and deep carmines are like the pink and red in the classical case of the Four-o'clocks was considered but found not to agree with the data from the crossings. Lastly some factor, also heterozygous, imparting the characteristic "Red McCormick" restriction of colour, seems at work among a majority of the light-coloured offspring. We may name this gene M and picture it as functioning only in the presence of D.

Do the data from the crossings sustain the genetic interpretation for

"Red McCormick" just advanced? The formula  $MmBbDd$  for "Red McCormick" is supported by the hybridization facts if the "Rurals" be looked upon as triply recessive, that is of formula  $mmbbdd$ . The eight possible "Red McCormick" gametic combinations are then determinative of the character and frequencies of the hybrid offspring:

- |                                   |                  |
|-----------------------------------|------------------|
| 1. $MBD$ —Red McCormick.          | 5. $MbD$ —Cream. |
| 2. $MBd$ —Dark, evenly coloured.  | 6. $Mbd$ —Cream. |
| 3. $mBD$ —Light, evenly coloured. | 7. $mbD$ —Cream. |
| 4. $mBd$ —Dark, evenly coloured.  | 8. $mbd$ —Cream. |

The number of plants with dark evenly coloured tubers actually observed fall somewhat short of expectation, but in other features the theoretic and actual agree rather closely.

It might be added that the self-pollination of "Red McCormicks" with male and female gametes of the eight kinds just enumerated leads theoretically to a group 27 Red McCormick : 12 dark, evenly coloured : 9 light, evenly coloured : 16 cream which does not seem a bad correspondence with the observed 8 Red McCormick : 2 dark, evenly coloured : 4 light, evenly coloured : 5 cream, considering the small numbers.

This interpretation of the behaviour of "Red McCormick" needs confirmatory data. If the assumption that the factor  $M$  acts only in the presence of the factor  $D$  be correct, then no plant with light uniformly coloured tubers should ever throw out "Red McCormicks," while of the deep coloured ones that have arisen in our pedigrees some should segregate out "Red McCormicks," and some of them should not.

TABLE I.

Parentage	I Red McCormick colour dis- tribution	II Medium to deep anthocyan (suffused)	III Very light to light anthocyan (suffused)	IV Cream	Total
Guarded selfings:					
Red McCormick—plant 5	7	2	4	3	16
" " " 41	1	—	—	2	3
Guarded crossings:					
Heavyweight 164 × Red McCor. 1	9	17	12	33	71
Heavyweight 161 × Red McCor. 9	6	8	6	29	49
Heavyweight 148 × Red McCor. 1	4	6	2	5	17
Heavyweight 135 × Red McCor. 48	2	2	2	4	10
Russet Rural 798 × Red McCor. 48	1	—	3	2	6
Russet Rural 484 × Red McCor. 10	1	—	—	4	5
Golden Rural × Red McCor. 9	2	4	—	7	13
Rural N.Y. 961 × Red McCor. 41	—	3	2	7	12
Unguarded crossings:					
Russet Rural 761 × Red McCor. 41	6	4	5	6	21
Heavyweight 45 × Red McCor. 48	—	—	—	1	1
Total for crossings ... ..	31	44	32	98	205





# INHERITANCE OF THE COLOUR PATTERN OF KING EDWARD POTATO.

By E. J. COLLINS,

*Botanist, The John Innes Horticultural Institution.*

KING EDWARD potato is well known from the typical distribution of pigment. It is splashed with pink<sup>1</sup>, the colouration being more or less confined to the eyes and to areas varying in size around the eyes. At the rose (distal) end of the tuber the pigmentation may become suffused over the whole area owing to the greater number and consequently closer approximation of the eyes. Colouration of the lenticels often occurs. On occasions, fully self-coloured tubers are produced, but the condition is not a stable one, for plants derived from such give tubers of the normal splashed type.

From the frequent presence of the pigmentation in the lenticels and eyes one is led to suspect a possible association between the respiratory activity and the production of pigment in these areas.

King Edward plants rarely flower at Merton, but in 1920 one plant among many developed two flowers. No pollen was produced but a cross was obtained by using the pollen of the variety Majestic.

Majestic is a variety with large white tubers which has been selfed and two batches of seedlings have been grown. In the first batch of 90 tuber-bearing seedlings, 87 bore white tubers; in the other 3 the tubers showed some traces of colour. In the second batch, 54 plants bore white tubers, and again 3 plants were observed which showed slight traces of colour in some of their tubers. In crosses with the following varieties, all having white tubers, Majestic being in each case the pollen parent, offspring with colour in the tubers have not been observed.

1923	1922	1921	1920
Snowdrop	Factor	Factor	Up-to-date
Bishop		Sharpe's Express	
May Queen		Witch Hill	
		President and reciprocally?	

In 15 other crosses, also with varieties bearing white tubers, the progenies have shown occasional seedlings with tubers bearing traces of

<sup>1</sup> Rouge 21, Code des Couleurs, Klincksieck et Valette.

## 202 *Inheritance of Colour Pattern of King Edward Potato*

colour. Such numbers as 1 to 104 white, 4 to 96 white, 9 to 31 white, 12 to 79 white, 13 to 77 white and 14 to 129 white have occurred. It has been noticed that the crosses which have produced the larger numbers of seedlings showing tubers with traces of colour, are those in which the white tuber of the parent is affected by light. In such forms as Epicure, British Queen, Favourite, Golden Wonder, etc., deep colouration with anthocyanin is developed in the tubers when exposed to light in addition to the normal greening. So much is necessary in explanation of the genetical constitution of the variety Majestic and it is being regarded here as a homozygous recessive white.

From the cross King Edward ♀ × Majestic ♂ ( $\frac{1}{2}\frac{1}{2}$ ) there were grown 92 tuber-bearing seedlings and of these, 46 produced white tubers and 46 carried tubers similar to the ♀ parent, King Edward. These figures represent undoubtedly a 1 : 1 ratio. In four plants bearing white tubers when the tubers became exposed at the soil level, pigment was developed, and in one plant with splashed tubers, exposed parts of tubers became self-coloured. Three only of the seedlings flowered during the season and these could not be carried over to the following season. In the next year, Seedling  $\frac{1}{2}\frac{1}{2}$ 45, which had tubers indistinguishable from those of King Edward and foliage very characteristic of that variety, flowered and was pollinated with Majestic pollen. From this cross ( $\frac{1}{2}\frac{1}{2}$ ) there resulted 46 tuber-bearing seedlings, 25 of which bore tubers similar in pigment distribution to King Edward and 21 bore white tubers, an approximation to the 1 : 1 ratio.

The absence of self-pinks in these progenies is noteworthy, for it might be expected that parti-coloured individuals selfed or crossed with a recessive white type would throw a proportion of fully coloured individuals. From the results obtained it is reasonable to assume that the parti-coloured pattern typical of King Edward depends on a definite factor and that it exists in the variety in a heterozygous condition, and that could it be obtained in a homozygous state it should breed true. Cf. picotee sweet peas and carnations. Further it should behave as a dominant to the recessive white and be recessive to full colour. On this assumption we have to deal with a multiple allelomorphic series in which homozygous self-red, parti-colour red, and white represent the respective terms.

# THE GENETICS OF THE WENSLEYDALE BREED OF SHEEP.

## I. THE OCCURRENCE OF BLACK LAMBS—AN EXAMINATION OF FLOCK RECORDS.

By F. W. DRY, M.Sc.

*Ackroyd Memorial Research Fellow, Leeds University.*

### PRELIMINARY STATEMENT.

A TYPICAL Wensleydale sheep is one with white wool and the skin of the face and ears deep blue in colour. In pedigree Wensleydale flocks, though black sheep are never used for breeding, a large number of black lambs are born. The actual proportion is about 15 per cent. These are the first two facts from which this enquiry starts. The position, as one learns it from the breeders, may be summarised as follows:

- I. There is a general belief that the production of blacks is associated with the blue complexion.
- II. It is hoped by throwing out blacks, as is always done in pedigree flocks, that the proportion of black lambs born will be reduced, or at any rate prevented from increasing.
- III. At the same time the general opinion is that blacks will always be produced.
- IV. It is known that in the families of two rams there is sometimes a wide difference in the percentage of blacks. This difference is thought to be due to an inborn difference between the rams.
- V. Most breeders have known or heard about a very small number of rams that were fathers of large families which did not include a single black, but these rams are regarded as very exceptional animals.

Fairly extensive data have been collected from Wensleydale breeders. The most important source of information is some flock records kept for 25 years. In a problem such as the present one the slow rate of reproduction of a large animal is the greatest difficulty, but these long

records go far towards disposing of this difficulty. We are further helped by having information from a large number of breeders, and it is especially useful to learn from two gentlemen who have kept flocks of black Wensleydales that when black animals were mated together all the lambs were black. On examining the facts recorded in the light of the well-known work on smaller animals we can show that there is a high degree of probability in favour of a very simple interpretation. This interpretation is, in fact, the simplest one possible. Supposing this to be correct, the conclusion which follows about each of the points listed above is as follows:

- I. Undoubtedly the throwing of blacks is associated with the blue complexion.
- II. If blacks were bred from, the proportion of blacks born would be higher, but white animals which have one black parent would not, on the average, produce more blacks than animals whose parents were both white, provided that each of the animals under consideration with both parents white was able to produce a black at all.
- III. If animals of both sexes with the deepest blue complexions are selected for breeding, blacks always will be produced. If the deep blue complexion were not insisted upon, blacks could be eliminated.
- IV. When white rams mated with white ewes sire any blacks at all, any difference in the percentage of blacks produced is not to be attributed to any inborn difference between the rams.

It is to be put down partly to chance and partly to a greater or smaller proportion of the ewes with which a ram is mated being incapable of producing blacks.

If this interpretation be correct we conclude at the same time that a considerable proportion of the ewes kept for breeding in Wensleydale flocks are unable to produce blacks.

- V. Fourteen Wensleydale rams used in pedigree flocks which never sired a black lamb have been reported. It is concluded that a great many ram lambs, and of course ewe lambs, too, which are incapable of producing blacks, are born in every flock, but that in the keen selection of the male animals for the blue complexion, very few such rams are selected for service in pedigree flocks.

## INTRODUCTION.

This account will show that black may be regarded as a simple recessive. Davenport<sup>1</sup>, discussing data given in Bell's *Sheep Catalogue*<sup>2</sup>, also found black to be recessive. Even at first sight the colour types in the Wensleydale breed call to mind the case of the Blue Andalusian. A deep blue complexion is the colour type aimed at in this breed, the colour being especially insisted upon in the head and ears, but the more blue is shown in other parts of the skin the better is an animal liked. It is the endeavour, therefore, to select blue-skinned animals for breeding. But "blue" sheep, in addition to throwing blacks, also give a number of lambs which are not blue enough for the standard of the breed. The term "pale" can be conveniently used for these. Some pales have a complexion definitely blue, but not deep blue, with the inside of the ears of a coppery colour. Occasionally the complexion is pink, without a trace of blue, but such animals are very rare. Between the deepest blue complexion and the completely pink all intermediate conditions can be found. Sometimes the ears are spotted or blotched in various ways. Often in a lamb which is born with ears blue in part and pale in part, the blue colour spreads until the ear is completely blue. Often, too, a lamb born with a pale blue complexion comes to have a deep blue one as it gets older. As one breeder said, "You do not know what a Wensleydale is, until it is a year old." It is thus not possible to draw a sharp line between "blue" and "pale," and not much will be said in this account about the proportion of pales. Sheep that are not black will often be spoken of simply as "white." The blacks, too, differ: some are thoroughly black, others are "silver-grey," having a mixture of black and white fibres which together produce a silvery appearance, but here again a complete series exists from deep black to light silver-grey. Often, part of an animal's coat would be described as black, part of it as silver-grey. There is also a change with age. Black and silver-grey sheep frequently appear lighter with successive shearings, and fibres have been found which are black distally, but in which the colour found gradually decreases in amount towards the proximal end, which is completely white. A black sheep will also sometimes have a little patch of white. Fortunately, however, we can say whether any animal is to be classified

<sup>1</sup> Davenport, C. B. (1915). "The Origin of Black Sheep in the Flock." *Science*, N.S. Vol. xxii, No. 569, p. 674.

<sup>2</sup> Bell, Alexander Graham (1904). *Sheep Catalogue of Beinn Bhreagh, Victoria Co., Nova Scotia*; showing the Origin of the Multinippled Sheep of Beinn Breagh and giving all the Descendants down to 1903, pp. 22. Washington.

as self-black or self-white. It is true that some white-woolled sheep have small patches of black, usually not more than a square inch or two, occasionally as big as one's hand, and, very rarely, even bigger than that; but of the large number of breeders consulted not one had ever seen a white sheep as much as a quarter black. We thus do not have to deal with a piebald condition. It should be added that, except when they are very young, black sheep appear dark brown because the tips of the fibres fade.

It is not possible to say whether the blue complexion accompanies a character of economic importance. While one is told that the blue face is the "beauty of the breed," it is also stated that the blue face is associated with a high proportion of lean to fat, a feature desired in the cross-bred offspring of Wensleydales. To investigate this question by experiment would be a big undertaking.

The data now to be presented have been collected in visits to many of the members of the Wensleydale Longwool Sheep Breeders' Association. The records of the flock kept by Lord Henry Bentinck, M.P., on the Underley Farm near Kirkby Lonsdale, are dealt with first. This flock was founded in 1897, and, unfortunately, dispersed in the autumn of 1922. Throughout the whole time it was under the charge of Mr G. Goland Robinson. It was one of the small number of famous flocks, which it is impossible for anyone outside, or I should imagine, inside the breed, to place in order of merit: it was also one of the largest flocks. Mr Robinson kept a record of the parentage, sex, and colour of nearly 3000 lambs born in the years 1898-1922. These records he very kindly put at my disposal, and in every way gave me all the help he could while I was working on them. By keeping these records Mr Robinson has in effect been conducting a large scale breeding experiment for 25 years.

That black is recessive is obvious, but the Underley records are the only data we have which throw light on the question of whether black is a simple recessive or whether matters are more complicated. As the percentage of blacks born in the Underley flock and elsewhere is definitely less than one in four, one might well suppose that black was a double recessive and that white animals of three genotypes existed able to produce blacks. The analysis of the Underley records, however, makes it probable that black is a simple recessive. If that be so the explanation of the fact of the proportion of blacks born being less than one in four will be that some animals are used for breeding, often a considerable proportion of the flock, which are incapable of producing blacks. After the data from Underley, the results of enquiry amongst other flocks are

given. At the time when the work in the field was being done Mr Robinson was Honorary Secretary of the Breeders' Association, and in addition to all his other help, he put me in touch with many of the members of the Association. The facts collected from all sources are then summarised and discussed.

#### DATA FROM THE UNDERLEY RECORDS.

For the whole period in which the flock was kept 3015 lambs are recorded born. For 60 lambs colour particulars are not given. These are of course omitted from the analysis. Some of these are lambs which were born dead or died soon after birth; in many cases they were in a condition in which the colour could not be recognised. For eleven lambs from seven matings the flock numbers of the ewes are not given, though the sires are recorded: these eleven lambs are included in calculating the percentages of colour types for the years in which they were born, and in calculating them for the individual rams, but they are left out in analysing the progeny of the individual ewes. Mr Robinson placed the lambs in three classes, pale, blue, and black, the pales being those which did not come up to what he regarded as the blue standard. The total number of rams used was 97. The number of ewes selected for breeding was 619. Three of these produced only dead lambs of which particulars are not recorded and one never had lambs at all. All the rest produced one or more lambs of which colour particulars were recorded. Ninety-five of the ewes were bought, mostly in the early days of the flock; the remainder, 524, were bred in the flock.

The number of females described as blue born up to and including 1920 was 895. As the flock was dispersed in the autumn of 1922, and as animals were not put to the ram until they were in their second year, the ewe lambs of the 1920 crop were the last from which a choice of breeding females was made. Of the ewe lambs written up as pale, nine were used for breeding, and five of them produced at least one black.

*The Sexes approximately equal in each Colour Class.*

*The percentage of each Colour Class.*

In each class the sexes are approximately equal as is shown in Table I, which also gives the percentages of the different colour types.

One ram, Lunesdale Quality 2032, sired 47 lambs which were all of them white. Every other ram that was the father of more than eight



## 208 *The Genetics of the Wensleydale breed of Sheep*

lambs had at least one black amongst his offspring. If the percentages of the colour classes are calculated with the lambs by Lunesdale Quality omitted, the figures are:

*Pale* 13·2 per cent.: *Blue* 66·0 per cent.: *Black* 20·8 per cent.

TABLE I.

Colour class	Male	Female	Sex not recorded	Total	Percentages
Pale	170	219	4	393	13·3
Blue	960	988	9	1957	66·2
Black	293	278	34	605	20·5
Totals	1423	1485	47	2955	100

### *Percentages of the Colour Classes for each Year.*

These are shown in Table II. It may be mentioned that if Lunesdale Quality's lambs, which were born in 1915 and 1916, are omitted the percentages of blacks for those two years become a little higher. We may conclude from this table that the proportion of blacks born is substantially as high at the end of the 25-year period as at the beginning. The figures

TABLE II.

### *Underley Flock. Percentages of the Colour Classes for each Year.*

Year	No. of ewes	Pale lambs		Blue lambs		Black lambs		Total lambs
		No.	%	No.	%	No.	%	
1898	4	1	—	3	—	5	—	9
1899	24	6	13	32	68	9	19	47
1900	32	9	16	30	55	16	29	55
1901	40	11	14	44	58	21	28	76
1902	37	12	16	49	65	14	19	75
1903	48	9	10	61	69	19	21	89
1904	70	25	18	82	60	30	22	137
1905	74	18	13	86	64	30	22	134
1906	83	32	23	80	57	29	21	141
1907	95	27	15	101	57	49	28	177
1908	99	28	16	114	64	35	20	177
1909	58	16	17	59	63	18	19	93
1910	82	8	6	99	77	22	17	129
1911	97	13	8	101	66	39	25	153
1912	92	27	16	110	65	32	19	169
1913	82	13	8	118	72	32	20	163
1914	70	16	12	94	70	24	18	134
1915	81	19	14	91	65	29	21	139
1916	82	24	16	97	66	25	17	146
1917	71	16	13	86	68	24	19	126
1918	78	17	13	90	69	24	18	131
1919	52	4	4	72	74	21	22	97
1920	68	6	5	88	75	24	20	118
1921	66	20	17	88	75	10	8	118
1922	69	16	13	82	67	24	20	122
Totals		393	13·3	1957	66·2	605	20·5	2955

for pales are certainly smaller in the second half than in the first, but as the difference is not large, and as there is no sharp line between pale and blue, it cannot be regarded as of very much significance.

*The Rams and their Progeny.*

Table III gives colour particulars of the lambs sired by each of the individual rams, 34 in number, whose families contained 30 or more lambs. Sixty-three other rams each sired a smaller number than 30. As has been stated, Lunesdale Quality was the only ram who sired any

TABLE III.

*Underley Flock. The Rams and their Progeny.*

Rams		Years when offspring were born	Pale lambs		Blue lambs		Black lambs		Total lambs
			No.	%	No.	%	No.	%	
Rams which sired 100 or more lambs									
Blue Beard 607	...	1901-04	17	12	93	66	31	22	141
Prince of Blues 973	...	1905-08	29	18	86	53	47	29	162
Leading Blue 1256	...	1908-09	28	23	74	60	21	17	123
Westmorland Blue 1300	...	1908-12	4	3	95	77	24	20	123
Royal Bertie 1728	...	1912-14	13	12	76	72	17	16	106
Underley Dreadnought 1968	...	1915-17	14	14	70	70	16	16	100
Prince Pippin 2396	...	1920-22	13	9	111	73	27	18	151
Rams which sired 50 or more lambs but less than 100									
Winder 523	...	1899-01	9	14	36	55	20	31	65
Welcome 893	...	1900-02, 1904	8	13	36	59	17	28	61
Moore's Blue 890	...	1904-05	8	14	29	51	20	35	57
Blue Hero 884	...	1904-06	9	15	43	70	9	15	61
King of Blues 1041	...	1906-08	14	18	47	60	18	23	79
Mowbrick Dreadnought 1480	...	1911	5	10	32	64	13	26	50
Underley Leader 1639	...	1912-13	8	14	38	68	10	18	56
Royal Derby's Heir 1731	...	1912-14	5	6	61	74	16	20	82
Royal Gotim 1948	...	1914-16	7	12	35	60	16	28	58
Royal Bertie's Hero 1830	...	1914	5	5	41	73	10	18	56
Drake 2236	...	1919	3	9	45	76	11	19	59
Bedale Blue 2691	...	1921-22	7	12	42	72	9	16	58
Rams which sired 30 or more lambs but less than 50									
Blue Stone 779	...	1903-04	5	16	20	62	7	22	32
Royal Maidstone 205	...	1904-05	7	15	29	62	11	23	47
Lucky Lad 884	...	1906	14	35	19	47	7	17	40
Lucky Jim 1045	...	1909-10	2	5	24	65	11	30	37
Prince's Pride 1371	...	1910-11	4	10	33	80	4	10	41
Blue Bertie 1314	...	1910-11	3	7	25	61	13	32	41
Royal Liverpool 1619	...	1911-14	5	12	23	55	14	33	42
Lunesdale Quality 2032	...	1915-16	10	21	37	79	—	0	47
Super Dreadnought 2306	...	1917-18	3	8	26	68	9	24	38
Royal Gotim's Heir 2178	...	1917	6	18	21	64	6	18	33
Slyne Minister 2302	...	1917-18	10	22	32	70	4	9	46
Gotim Yet 2367	...	1918-19	2	7	19	63	9	30	30
Admiral Drake 2327	...	1918-19	1	2	31	72	11	26	43
Cock of the Walk 2566	...	1921	11	22	35	71	3	6	49
Duke of Underley 2861	...	1922	4	13	21	68	6	19	31

60 other Rams each sired less than 30 lambs

considerable number of lambs which did not contain a black. It was possible to follow up the rest of the career of this ram, who did not die until 1922. He sired well over 200 Wensleydale lambs and quite a number of cross-breds, but every lamb by him was white.

The rams which did sire blacks had percentages of black offspring in their families varying from 35 per cent. (on a total of 57) to 6 per cent. (on a total of 49). Do these figures indicate that in factors concerned in the production of black the rams are of more than one genotype, or are we to suppose that we have to deal with only a single factor, and that the differences in the proportion of blacks in the families of the different rams are due to chance fluctuation and partly, too, to some rams having been mated with a larger proportion of ewes homozygous for the factor which causes an animal to be not black, but white? We cannot decide this question definitely from the figures in Table III. Either explanation might be true. It will be seen, however, that only three rams have a percentage of black lower than 15 per cent. These rams are:

Prince's Pride 1371, with 4 blacks in 41 (10 per cent.);

Slyne Minister 2302, with 4 blacks in 46 (9 per cent.);

Cock of the Walk 2566, with 3 blacks in 49 (6 per cent.).

On examining the records of the families of these rams, it is found that for the last two rams named an unusually large proportion of their lambs were from ewes which, whether they were capable of doing so or not, never produced any blacks. Slyne Minister had 24 such lambs, and Cock of the Walk 25. We cannot come to any definite conclusion from the examination of Table III, but the interpretation that the black producing rams are all of one genotype, that is to say, that black is a simple recessive, is, from the figures given in this table, somewhat more probable than any other.

Differences are to be seen in the percentages of pales, Westmorland Blue 1300, having strikingly few pales, only 4 in 123. Whether this low proportion is to be attributed to chance or to the occurrence of one or more modifying factors we cannot say.

#### *The Ewes and their Progeny.*

Colour details of the offspring of each individual ewe were compiled from Mr Robinson's records. We are only concerned now with whether a lamb was white or black. As must inevitably happen, a great many families did not include a black, for the average size of a family was about five. The largest family numbered seventeen, though it happens

that this family, for a reason to be explained directly, was omitted from the analysis of the families. The method used is that employed by Jones and Mason<sup>1</sup>, who referred to an unpublished MS. by Sewall Wright. This is a method for comparing the figures recorded with the figures expected if black is a simple recessive. The figures expected are calculated from a modified Mendelian ratio which allows for the omission of all families not containing any black lamb. For the purpose of this analysis all lambs by Lunesdale Quality are regarded as non-existent. All families are omitted which contained one or more lambs by one of the rams which sired a small number of lambs which were all white. And finally the eleven lambs from seven matings, where the flock numbers of the ewes

TABLE IV.

*Underley Flock. Analysis of the families of the ewes which produced at least one black.*

Size of family	No. of families containing blacks	No. of blacks	Expected if black be a simple recessive	
			Calculated proportion of blacks	Calculated no. of blacks
1	5	5	1.0000	5.00
2	37	45	.5714	42.28
3	44	64	.4324	57.08
4	44	62	.3657	65.36
5	36	58	.3278	59.00
6	41	73	.3041	74.81
7	35	85	.2885	70.68
8	27	63	.2778	60.00
9	20	34	.2703	48.65
10	12	28	.2649	31.79
11	10	27	.2610	28.71
12	6	18	.2582	18.59
13	3	9	.2561	9.99
14	3	10	.2546	10.69
16	2	11	.2525	8.08
<hr/>				
			592	590.71

could not be ascertained, are omitted. Jones and Mason were studying the inheritance of congenital cataract, which they believe to be inherited as a simple recessive. They say:

The proportion of recessives varies according to the number of children in the family and ranges for a three-to-one ratio, from 100 per cent. in families with one child to very nearly 25 per cent. in families with 15 children. The proportion is calculated from the formula

$$X = \frac{1}{4 \left[ 1 - \left( \frac{3}{4} \right)^n \right]},$$

where  $n$  is the number of children. Since the criterion for including any

<sup>1</sup> Jones, D. F. and Mason, S. L. (1916). "Inheritance of Congenital Cataract." *Amer. Nat.* Vol. L, p. 119.

## 212 *The Genetics of the Wensleydale breed of Sheep*

family is the production of one abnormal child, all families with one child must have 100 per cent. abnormal children. The proportion decreases, according to the law of chance, as the number of children increases, finally reaching 25 per cent. as the number of children becomes large.

The result of this analysis of the families of the ewes is given in Table IV. The calculated total number of blacks is 591, the actual figure is 592. In the families which contained seven lambs the average number of blacks is appreciably higher than expected; in those which contained nine lambs the actual number is appreciably lower than the calculated number. For the rest of the table the numbers realised and those calculated agree closely.

### INFORMATION FROM OTHER SOURCES.

#### *Proportions of the Colour Classes.*

The percentage of blacks can be calculated from figures which have been collected from other sources; these are given in Table V. Mr John Allison, of Howgrave Hall, kindly lent me the flock records of the late Mr John H. Calvert. These records are not extensive enough to make it worth while to analyse the families of the individual ewes. Mr W. S. Heslington of Ripon has kept records of the total number of lambs born and the number of blacks for eleven years, but did not keep a detailed record of the parentage of each animal. The figures given from various breeders for 1921 and 1922 do not include the offspring of a few rams known not to sire blacks.

TABLE V.

	Period	Total	No. of blacks	Percentage black
Mr John H. Calvert	1895-1900	122	29	24.0
Mr W. S. Heslington	1912-1922	436	72	16.5
Various breeders	1921	965	140	14.5
" "	1922	1313	202	15.4
" "	1923	899	144	16.0

These figures include some from flocks in which less rigid attention is paid to the blue complexion than in the Underley Flock. It will be seen that the percentage of blacks calculated from the data from these various sources is appreciably less than the Underley percentage.

Some information, too, was collected about the proportion of pales, but in distinguishing pale from blue individual opinion plays a large part. It is however safe to say that the proportion of lambs which breeders call pale is less than the proportion of blacks.

A small number of breeders have told me that they think the proportion of blacks born in their flocks is less than formerly, but the majority who expressed an opinion believe the proportion to be as large as ever it was. But several breeders have stated that the proportion of pale colour misfits is smaller than formerly. This is, I believe, the opinion of the majority, and it is a view to which the Underley figures lend a little support.

*Rams whose offspring are all white.*

The case of Lunesdale Quality has already been given. Thirteen other rams whose offspring did not include a single black lamb have been heard of, and some of them examined, but not many are now living. The names of the rams are: Bluebottle 1582, Brewer 612, Hercules 98, Hoggarth's Champion 2749, Holme Fashion 2133, Lucky Pearl 1353, Lundholme Pippin II 2617, Middleton Bentinck 2480 (son of Lunesdale Quality), Middleton Coalition 2624 (son of Middleton Bentinck), Mount Pearl 2628, Royal Salute 2658, X-Rays 2239. One other ram, used about 25 years ago, was mentioned to me, but his name was not remembered. Some of these rams, such as Brewer and Royal Salute, sired a couple of hundred lambs; the remainder were each credited with more than fifty. Of some of these rams which are dead it is said that they were typically blue, but others have been described by those who had used them as pale. The five rams with an all-white record which I have seen were very fairly blue on the face, head and outside of the ears, but the inside of the ears was in every case of a coppery colour; it was not blue.

The question presents itself, are there more pales than usual in the offspring of these rams? As has already been said, where a distinction has to be made between pale and blue, personal opinion must play a large part. In a number of cases, in some of which I have seen the lambs, it can certainly be said that the proportion of blue lambs out of the total born is higher than the average for rams which do sire blacks. This, it will be seen in the Underley ram list, is true for Lunesdale Quality. But in other cases breeders have told me that the proportion of pales was high.

*Black mated with Black.*

On the result of mating black with black information was given by two gentlemen who have had black Wensleydale flocks, one quite large, the other small, which were kept in parks for what might be called ornamental purposes.

Mr E. G. Howsin, estate agent on the Huntroyde Estate at Padiham,

near Burnley, told me that a flock of black Wensleydales was started there about 1906. The flock was disposed of about 1916. The original sheep all came from pedigree flocks, and others were added from time to time, always from pedigree flocks, every animal being thoroughly black. Some of the rams used were bought from pedigree flocks, others were bred. In the later part of the time the flock was divided into two halves and a ram lamb bred in one half would sometimes be kept for use in the other half. The number of rams used during the 10 years would be about 10 or 12. The usual number of ewes was 40. Mr Howsin estimates the number of lambs born in the 10 years as 600. Every lamb was black; only a single individual was of a colour that fell short of typical black and that was of a "dirty brownish greyish" colour. It was, he said, of a self colour, the coat did not consist of white fibres intermingled with black. A few lambs had just a little white in them, but never a patch "bigger than one's thumb."

Mr F. Samuelson, of Breckenborough Hall, near Thirsk, had lambs by a black ram from a small number of black ewes in 1916 and 1917. These sheep were got together by Mr J. W. Greensit, of Holme-on-Swale, who states that they were jet black, without, so far as he had observed, any white fibres, though it is of course difficult to be sure on this point. In Mr Samuelson's records 16 lambs are listed, all black. The lambs were all thoroughly black except one that was silver-grey round the base of one ear.

*Black mated with Blue.*

Owners of registered flocks are prohibited from breeding from blacks, but a few people have told me that they had done so before joining the Sheep Breeders' Association, or, outside their pedigree flocks, breeders have occasionally put black ewes to the ram. Some people who do not belong to the Association have bred from blacks recently. But it was not possible to obtain precise information about the results of this mating. Mr J. T. Camplin, of Newby, near Penrith, said that in his experience two-thirds of the lambs had been black. Several people said they had had more whites than blacks. But these statements were all based on a small number of lambs and no records had been kept.

With regard to the colour of the lambs born in this mating, the majority were said to be typically blue, but Mr J. T. Halliday, of Whitrigg, near Penrith, said he had bred a few pale blue lambs from black ewes. Mr Camplin, too, told me of one such lamb which was pink-faced at birth, with pink ears with darker reddish spots on them;

at six months this lamb was a little blue in the face and ears, but, he said, it was lacking in colour and never would have acquired the proper colour; at that age he disposed of it.

*The Keen Selection of Stock Rams.*

It is obvious that the proportion of ram lambs born in any breed which come to be used for breeding in pedigree flocks must be a very small one. It is true that in the Wensleydale breed, as no doubt happens in many breeds, the greater part of the stock rams are bred by quite a small number of prominent breeders. The 1921 Flock Book was examined with this point in mind. One hundred and twenty-five rams are registered in that Flock Book; 76 of these rams, one of them known to have produced only white lambs, were bred by twelve well-known breeders, nobody else having bred more than two rams which were registered in that book. In 1920 these breeders had put 581 ewes to the ram. At a moderate estimate these twelve breeders would rear 250 male lambs each year. Even in these flocks, therefore, the rams sold for pedigree breeding are selected from a much larger number of animals born.

*Black lambs in Wensleydale-Scotch Mountain crosses.*

I have been told that a few black lambs are born in the cross of Wensleydale ram on white Scotch Mountain ewes, but they are said to be very rare, even though the Wensleydale used be a black, as he quite often is. In the second cross, when gimmers born in the first cross are put to a white-woolled Wensleydale ram, blacks are common. One gentleman who bred a large number of animals in this second cross estimated his blacks at an average of 7 per cent.; another said that he gets up to 10 per cent. The latter stated that he used one pale blue Wensleydale ram for the second cross for 14 years and that this animal did not sire a single black.

*The Blue Face in the Early Days of the Breed.*

It is often suggested that blacks were used for breeding in the early days of the breed, and that the use of blacks helped in establishing the blue type. From what breeders have told me we may be quite sure that to some extent blacks were bred from, but to what extent it is impossible to say. The Wensleydale breed was founded in comparatively recent times. A full account of its history has just been published by H. G. Robinson<sup>1</sup>. The main facts about colour which are of interest for

<sup>1</sup> Robinson, H. G. (1923). "Wensleydale Sheep." *Journ. of the Royal Agricultural Society of England*, Vol. LXXXIII.



our present purpose are also given in the introduction of the first volume of the Flock Book on the "Origin, Early History and Properties of the Wensleydale Longwool<sup>1</sup>." The following paragraph is made up mainly of extracts from this paper.

All authorities unite in asserting that the Mugs—the old name for Wensleydales—were a branch of the old Teeswaters, a breed which sprang from the districts lying along the banks of the Tees, in the county of Durham. It is stated that about 1840 the head and ears of these sheep were white with black spots. As was the case with almost every other long-woolled breed of sheep, a dash of the improved Leicester blood was introduced to remedy the defects of the breed. The Leicester blood was infused through Bluecap, a celebrated ram belonging to Mr Richard Outhwaite. He was from a Mug or Teeswater ewe and sired by an elder Bluecap, bred by the famous Leicester breeder, Mr Sonley, of Lund Court, Kirkby Moorside. This ram was probably the finest ever bred by Mr Sonley, and, from a painting still in existence, he appears to be of enormous size and substance, and to have a fine blue head. Mr Outhwaite's Bluecap was also a very striking animal, and allowed to be the best ram in the North of England in his day. He had a very dark blue head, and his skin was nearly black, although covered with fine white lustrous wool. Besides using him on his own flock of Mugs, Mr Outhwaite let Bluecap out to other breeders. Captain Harcourt of Swinton Hall, near Masham, used him to his Mug ewes, and in turn, sons of Bluecap were used by other breeders. Bluecap was lambed in 1839, and the ten following years, were without doubt, the most important in the history of the breed. The most striking feature about Mr Outhwaite's Bluecap was the deep blue tinge about his head, ears, and skin. This property was transmitted to many of his offspring from spotted faced Mug ewes, and it was soon discovered that the rams with blue heads begot lambs from Blackfaced ewes of a darker grey colour about the face and legs. This being a desirable property in the cross-bred offspring, the blue face became a point to be aimed at by ram-breeders and soon became a distinct feature of the breed.

#### SUMMARY.

The chief points established may be summarised as follows:

(I) The proportion of blacks born in the Wensleydale breed is not getting less, although blacks are not used for breeding.

<sup>1</sup> *Pure Select Wensleydale Flock Book*, Vol. 1, 1890.

(II) The proportion of blacks born is something like 15 per cent. It is definitely less than 25 per cent.

(III) When black is mated with black all the offspring are black.

(IV) Some rams produce offspring which are all white.

(V) The results of the analysis of the families of the individual ewes in the Underley flock is closely in accord with expectation on a one-factor interpretation.

We may therefore conclude with very fair confidence that the simplest possible interpretation is correct, namely, that black is a simple recessive. We cannot be certain that it is not a double recessive, but this is improbable. The blue or pale blue Wensleydale is clearly a dominant white. We may regard it as a suppressed black, or a black in which the colour is restricted to the skin. In Mr Robinson's words, "The blue Wensleydale is a black sheep with a white coat." And in this connection we may recall the frequent occurrence of a little patch of black in the wool of a white Wensleydale. We must conclude that rams whose offspring are all white are homozygous for the dominant white factor; that the bluest Wensleydales of all are probably all heterozygous and that some which are only fairly blue are also heterozygous. We must conclude, too, that a great many homozygous animals are born and that on the average they are not so blue as the heterozygotes; of the homozygous males only very few can be selected for stock rams in pedigree flocks. But of the homozygous females we must believe that a great many do become breeding ewes, for the proportion of blacks born in the breed, after we exclude the offspring of rams known not to produce blacks, is still clearly less than one in four. But as in a Wensleydale flock the ewes are not mated with blacks, even the largest families are not big enough for us to know whether any particular ewe which never has a black is capable of producing one. From the Underley figures the conclusion may be drawn that even in that flock about one in six of the ewes were homozygotes. For the whole breed the proportion is probably two in five.

While it may well be that those animals which are the very bluest inside the ears are all heterozygotes, there are several further points—assuming our interpretation to be correct—which emphasise the difficulty of distinguishing between homozygote and heterozygote in cases where the animal is not one of the bluest. We may be sure that in the Underley flock the number of homozygotes born was much greater than the number of animals which Mr Robinson described as pale. Again, of the

offspring of such rams as Lunesdale Quality and Royal Salute, more than half would be expected to be homozygotes, but less than a quarter were regarded by the breeders using them as pale. And it may also be mentioned that some of the lambs bred in the mating Black by Blue, lambs which on our interpretation must be heterozygotes, were reported to be pale.

Other questions present themselves which cannot be answered by an examination of the data collected. What is the difference between deep black and silver-grey? What is the difference between a sheep with a pale blue complexion and an animal with a pink one? Explanations can easily be suggested, but these are matters for experiment.

In a further contribution it is hoped to give the results of breeding experiments which are at present in progress.

SEX RATIO DATA FOR TWO CHALCID EGG  
PARASITES OF THE COFFEE BUG  
(*ANTESTIA LINEATICOLLIS*)

By F. W. DRY, M.Sc.

*Ackroyd Memorial Research Fellow, Leeds University.*

THE two common species of the egg parasites of the Coffee Bug (*Antestia lineaticollis*, Stal.) in Kenya Colony were named by Dodd<sup>1</sup> *Hadronotus antestiae* and *Telenomus truncativentris*. In a bulletin on the Coffee Bug (Anderson<sup>2</sup>), and in a paper on the egg parasites (Dry<sup>3</sup>), something has been said about the sex ratio for these parasites. The present object is to give in detail the figures which were obtained. For each species the figures are given first from collections of Coffee Bug eggs from the field, then from parasite life-history work. As is not uncommonly found with hymenopterous parasites the females were much more numerous than the males in the offspring from mated females, while in asexual reproduction all the offspring were males. The proportions of the sexes are much the same as those found by McColloch and Yuasa<sup>4</sup> for the Chinch Bug Egg Parasite (*Eumecrosoma benefica*, Gahan). Of 4474 parasites which they bred in the laboratory from mated females, 1297, or 29 per cent., were males, while unmated females always produced males. Out of 465 parasites reared from eggs collected in the field the number of males was 132, and they point out, therefore, that sexual reproduction appears to be the rule in the open.

Coffee Bug eggs which have been parasitised can be distinguished by the fact that after about a third of the time of the parasite life cycle within the host egg has been passed through, this egg changes from white to a darker colour, usually bluish-grey. This colour change does not take place in eggs which have not been parasitised; a few days before the young bugs hatch the eyes and tylus of the nymph show red through the cap of the egg. The length of the Coffee Bug egg stage is a third, or

<sup>1</sup> Notes on the Exotic Proctotrupoidea in the British and Oxford University Museums. *Trans. Ent. Soc. Lond.*, Jan. 1920.

<sup>2</sup> "The Coffee Bug." *B.E.A. Dept. of Agric., Div. of Ent.*, Bull. I, 1919.

<sup>3</sup> "The Egg Parasites of the Coffee Bug (*Antestia lineaticollis*, Stal.) in Kenya Colony." *Bull. Ent. Research*, Vol. XII, part 2, Sep. 1921.

<sup>4</sup> "Further Data on the Life Economy of the Chinch Bug Egg Parasite." *Journ. of Economic Entomology*, April 1915.

less, of that of the life-cycle of the parasite within the egg. The Coffee Bug eggs are laid in clusters, usually of ten, eleven, or twelve. The eggs collected in the field were kept under observation and those which turned blue separated from the others. In due course most of the blue eggs yielded parasites, and some were obtained, too, from some of the small numbers of eggs which became only dirty white in colour, but the majority of such eggs produced nothing. There was no indication that a Coffee Bug egg ever produced more than one parasite, and during the work many hundreds of eggs were kept singly in small tubes. The parasites hatching were preserved and the numbers of each sex of each species counted. All but a few collections yielded both species, but as we are not concerned now with the proportions of the two species, the data for *Hadronotus* and for *Telenomus* from the same collection are given separately. Where the number of one parasite species from a collection reached 200 the figures for that collection are stated. If the number was less than 200 details are not given, the figures from the smaller collections being combined. For each species the figures are given first for collections of eggs from a coffee field of about six acres on the Government Farm at Kabete. From this field a considerable number of collections were made between July 1917, and September 1920. After this the records are given from collections from other plantations from each of which usually only a single collection was made.

In the life-history work the female had never been exposed to a male when the experiment was started, except in Series Z for *Telenomus*. In those experiments where reproduction was asexual the females throughout their lives were kept away from males. In experiments with mated females, except in the series just named for *Telenomus*, the act of copulation was observed, this taking place not many minutes after the parasites were introduced to each other. The parasites were kept in tubes plugged with cotton wool, and were provided with a pad of cotton wool which was kept moist, with water in some experiments, with dilute golden syrup in others. Coffee Bug eggs which had been deposited in the laboratory and protected from parasite attack were placed in the tubes in little cork trays. Where water only was provided the eggs were never changed; where dilute golden syrup was given fresh Coffee Bug eggs were substituted every three days so long as a female parasite lived, except in the last *Telenomus* series. In the work with unmated females of both species some were given water, some dilute golden syrup, but the sex of the offspring being always the same, details of the different experiments are not given. In Series A of both species,

where the females were mated, both parents had emerged and been seen to mate the day that the experiment was set up, neither having mated with any other individual; after mating the pair were placed in a tube where they remained the rest of their lives; they were given water. In Series B of both species the age of one or both of the parents was a little more than one day when mating took place, or else, with *Telenomus*, the male might not be put in the tube with the female, and the same male might mate with the females of more than one experiment; the parasites were provided with water. In Series C of *Hadronotus* the same male, who was not even given water, and lived for five days, mated during this time with sixteen virgin females. With the first two he mated twice, with the others once. Each female was mated and then placed alone in a tube with eggs on the day on which she emerged, and was kept supplied with water. *Telenomus* males were got to mate with two, three, or four females, but males of this species were never given the opportunity to mate with any number much larger than four. In the one experiment, D, for *Hadronotus*, a mated female was fed on dilute golden syrup. The conditions in Series Z of *Telenomus* will be described a little later.

### *HADRONOTUS* INTESTIAE, Dodd.

#### 1. Data from collections of Coffee Bug eggs from Coffee Plantations.

Place	Date	Total	Number male	Percentage male
Kabete, Government Farm field	1917, July	481	119	25
" " "	Aug.	573	168	29
" " "	Sep.	415	129	31
" " "	Oct.	627	175	28
" " "	1918, Oct.	247	58	23
" " "	Nov.	355	68	19
" " "	Dec.	322	66	20
" " "	1919, Jan.	458	75	16
" " "	March	589	107	18
" " "	May	231	35	15
" Smaller collections	... ..	879	168	19
Totals for Kabete, Government Farm field		5177	1168	22.6
Other plantations				
Kabete ... ..	1917, July	438	93	21
Kabete ... ..	1918, Sep.	390	78	20
Ruiru ... ..	Sep.	268	48	18
Smaller collections ...	1917, July to 1920, May	729	177	24
Totals for other plantations ... ..		1825	396	21.2
Totals from all sources ... ..		7002	1564	22.3

2. *Data from life-history work.*

(1) *Parthenogenetic reproduction.* From 29 unmated females 770 offspring were obtained. They were all males. In these experiments 64 other Coffee Bug eggs went blue from which parasites did not emerge.

(2) *Sexual reproduction.* From 42 mated females 921 offspring were obtained, of which 189, or 20.5 per cent., were males. In these experiments there were 65 other Coffee Bug eggs which went blue but from

	Lab. No. of female	Total offspring	Male offspring
Series A.	165	24	3
	178	18	6
	202	20	5
	203	16	5
	204	25	2
	205	34	0
	206	25	8
	212	29	4
	213	21	6
	214	21	4
		— 233	— 43
Series B.	109	16	3
	110	19	4
	111	21	6
	112	11	5
	136	11	3
	144	19	6
	145	16	2
	146	25	7
	151	19	3
	157	14	1
	158	25	8
	159	22	7
	164	17	5
	208	21	4
	209	16	3
		— 272	— 67
Series C.	160	28	5
	161	24	1
	162	22	4
	166	23	1
	167	23	3
	168	22	7
	169	18	6
	170	19	5
	171	26	2
	172	18	3
	173	26	9
	174	28	3
	175	26	6
	176	26	9
	177	26	2
	183	14	2
		— 369	— 68
Experiment D.	3001	47	11
		— 47	— 11
Totals	...	921	189

On these figures the percentage of males is 20.5.

which parasites did not emerge. The proportion of eggs to which this happened was thus not more than in the experiments where reproduction was asexual. In both cases a small number of the Coffee Bug eggs used did not go blue and did not hatch Bug nymphs; the numbers of such eggs were not recorded but the proportion was certainly small and not very different from the proportion of Coffee Bug eggs laid in the laboratory and not exposed to parasites which failed to hatch. These facts, therefore, do not point to a higher mortality amongst immature males than amongst immature females.

In some of the experiments the males used as parents had been bred in the laboratory from virgin mothers. It is of course very possible that whether a female has been mated or not, her male offspring always develop from eggs which have not been fertilized. The proportions of the sexes in the offspring of females mated with males bred from virgin mothers were similar to those in the offspring of females whose mates were either known to have had mated mothers or were reared from eggs from the field. The females in question, seven in number, produced 154 offspring, 31 of them, or 20 per cent., males. One of these seven females, it happened, was the only one out of the 42 bred from in all which produced only females; they numbered 34.

The figures from each of the 42 individual females are given above.

*TELENOMUS TRUNCATIVENTRIS*, Dodd.

1. *Data from collections of Coffee Bug eggs from Coffee Plantations.*

Place	Date	Total	Number male	Percentage male
Kabete, Government Farm field	1917, July	532	128	24
" " "	Aug.	280	62	22
" " "	Sep.	276	70	25
" " "	Oct.	328	107	33
" " "	1918, April	271	45	17
" " "	July	276	86	31
" " "	Sep.	275	61	22
" " "	1919, Jan.	247	47	19
" " "	1920, Jan.	528	98	17
" " "	May	260	57	22
" " "	July	346	70	20
" Smaller collections ...	... ..	954	248	26
Totals for Kabete, Government Farm field		4573	1079	23.6
Other plantations				
Kyambu ... ..	1917, Aug.	204	41	20
Thika ... ..	Dec.	371	107	29
Kabete ... ..	1918, Feb.	254	72	28
Limuru ... ..	March	248	70	28
Kyambu ... ..	March	255	45	18
Limuru ... ..	May	282	70	25
Kabete ... ..	Sep.	208	68	33
Ruiru ... ..	1920, Jan.	339	110	32
Limuru ... ..	May	485	130	25
Smaller collections ...	1917, July to 1920, May	1138	311	27
Totals for other plantations ...		3784	1024	27.1
		8357	2103	25.2



2. *Data from life-history work.*

(1) *Parthenogenetic reproduction.* From 29 unmated females 322 offspring were obtained, all males.

(2) *Sexual reproduction.* In Series A 10 mated females produced 75 offspring, 46 being males. In Series B 9 mated females had 75 offspring, 41 of them males. In these experiments, therefore, more than half (87 in 150) of the parasites bred were males. As already mentioned, the act of copulation was observed in every case. At least one female was produced in all but three experiments; in these three the numbers of males were 2, 5, and 9. The conditions evidently did not suit the parasites. Water, but no food, was supplied. In the experiments with unmated females those provided with dilute golden syrup produced considerably more offspring than those only given water. In the three experiments of Series Z, where the conditions were different, the ratio expected in the light of the facts which have been recorded above was obtained. Three *Telenomus* females emerged from Coffee Bug eggs in the presence of males about a day older than themselves. After all these parasites had been together about a day the females were taken out separately each accompanied by one of the males. Each pair was put into a tube with 30 bug eggs and provided with dilute golden syrup until death. The act of copulation was not observed. The offspring produced by these three females were the following:

	Lab. No. of female	Total offspring	Male offspring
Series Z.	401	17	1
	402	12	1
	403	21	8
Totals	...	50	10

In this series just one-fifth of the parasites bred were males.

## CONCLUSION.

Both *Hadronotus* and *Telenomus* can reproduce asexually, all the offspring so produced being males, but we may conclude that in natural conditions mating is the rule, and that in the offspring of fertilized insects the females regularly outnumber the males by three or four to one. These parasites thus present an interesting problem in sex determination. They are easy insects to work with, for both of the parasite species and their host breed all the year round in the Highlands of East Africa, and the Coffee Bug readily lays eggs in confinement. Unfortunately it is not at present possible for this work to be continued by the Kenya Division of Entomology.

## GENETICS OF THE JAPANESE RABBIT.

By W. E. CASTLE.

*Bussey Institution, Harvard University.*

THE so-called "Japanese rabbit" is a variety of the domestic European rabbit which has a yellow coat mottled with black. There is no more reason to suppose that it originated in Japan than there is to suppose that the Himalayan rabbit originated in the Himalayan mountains, or the Abyssinian cavy in Abyssinia. It is one of those outlandish names so commonly employed by breeders of pet-stock to obscure the origin of a novelty and throw competitors off the trail of duplicating its production. I have inquired of several Japanese scientists as to their acquaintance with the variety but all have disclaimed any knowledge of it.

The variety was apparently unknown in England in 1889 when K. W. Knight edited a revised and enlarged edition of *The Book of the Rabbit*. Had its existence been known at that time, the fact would doubtless have been mentioned.

In Rayson's *Rabbits for Prizes and Profit* (undated edition, probably issued about 1900), the variety is figured and described and its introduction to the notice of English fanciers is dated back to 1893, "when a short notice of it was published in a fancy paper." C. H. Lane in *Rabbits, Cats, and Cavies*, London, 1903, mentions the same date, probably taken from the same source. In Rayson's book we read "In the summer of 1895 the writer paid a special visit to the Jardin d'Acclimation, in Paris, with the object of acquiring the best possible specimens of the breed, the French Zoo being the only place where they could then be obtained." This statement would lead one to believe that the variety probably originated in France within the last half-century and was first introduced into England about 1895.

The brindled or mottled coat, the distinguishing character of this variety, is similar in appearance to the tiger markings of Great Dane dogs and Boston bull terriers, to the brindled coat of the tortoise-shell guinea-pig, and the occasional brindling of cattle. It is inherited also in similar fashion. It is a simple Mendelian unit-character, an allelomorph of yellow and of black respectively, dominant over the former, recessive to the latter. This is exactly parallel with the condition in

guinea-pigs first clearly worked out by Ibsen, in which tortoise-shell is dominant over yellow and recessive to black.

The peculiarity of the case of the Japanese rabbit lies in its relation to the agouti or gray factor.

In guinea-pigs it is difficult or quite impossible by inspection to distinguish a yellow individual which possesses the agouti factor from one which does not possess it. But in black pigmented individuals the agouti is readily distinguished from the non-agouti by the banding of the hairs, the agouti individual having hairs tipped with black, below which comes a band of yellow, and then the basal part of the hair is of dull black. In the non-agouti individual, no yellow band is present, so the hair is black throughout its length.

In rabbits the agouti and non-agouti yellows are different in appearance, the latter being "sooty" even on the belly and under surface of the tail, which are clear white in an agouti yellow rabbit. The same difference is recognizable in the *yellow areas* of Japanese rabbits which *do* possess the agouti factor, as compared with those which do not. The former have white bellies, the latter do not. But in the *black patches* of their coats, agouti and non-agouti Japanese rabbits look alike. In neither sort is the black hair banded with yellow, though the yellow areas of the coat usually indicate clearly whether the agouti pattern is present or not. In doubtful cases demonstrative evidence may be obtained by mating the Japanese rabbit with an ordinary black one. If the Japanese parent carries the agouti factor, gray young will be produced; if not, black young. From these facts it is clear that the agouti factor has no effect on the *black* hairs of a Japanese rabbit, though it does on the yellow hairs. How are we to account for this?

Punnett was the first to show that there are two different kinds of black rabbits, dominant and recessive blacks<sup>1</sup>. In the former the agouti factor produces a scarcely perceptible ticking of the hair, or no ticking at all, whereas in ordinary (recessive) black rabbits presence of the agouti factor turns the coat into an ordinary gray (agouti). Yellow behaves as the allelomorph of either kind of black, and they as allelomorphs of each other. Yellow will always show the presence of the agouti factor by the white ventral surface and the absence of sootiness from the fur. Recessive black will also show the presence of the agouti factor by the yellow banding of the fur making the coat gray and the ventral surface whitish as in yellow agouti rabbits.

<sup>1</sup> Mr Feldman has recently shown that in *Mus rattus* also there occur both a dominant black and a recessive black variety.

If we could produce a rabbit part of whose coat was yellow, the other part being recessive black, and if we should then bring into it the agouti factor, the coat would become *gray-and-yellow*, with a white belly. Now in the Japanese rabbit, the black never becomes gray through the action of the agouti factor; it remains unticked black, *even on the belly*. This shows that the black of the Japanese rabbit is *dominant* black, and this in turn throws an interesting side-light on the origin of mottled animals and plants. The mottled condition is transmitted in a single gene and is in reality a mosaic of what are ordinarily distinct and alternative genetic states. The mottled guinea-pig is produced by a gene which is a mosaic of yellow and of recessive black (which the agouti factor will convert into gray). In the guinea-pig no other kind of black is known. But in the rabbit we have both dominant and recessive black. The agouti factor will change the latter to gray but not the former. The Japanese rabbit has arisen as a mosaic of yellow and dominant black, probably through incomplete separation of the two at the reduction division in a heterozygote between the two.

What about the commonly assumed simple and changeless character of genes? We may, if we choose, avoid the difficulty, as others have done, by calling this mosaic a "mutation."

#### THE EVIDENCE.

In May 1920 I obtained from a dealer two New Zealand Red does which had been bred to a Japanese buck. The New Zealand Red is a large variety of rabbit with an intense reddish-yellow coat, showing the presence of the agouti factor by the white belly and tail. Early in June the does gave birth to good sized litters, one consisting of six, the other of nine young, all with the characteristic Japanese mottling of black and yellow. The sire of these fifteen young was clearly homozygous for the Japanese pattern which he transmitted to all of the young. The Japanese character was also clearly dominant in every case over the self yellow condition of the mothers. One of the mothers was subsequently mated to self yellow bucks producing in each case only self yellow young. Hence the Japanese character of the two litters mentioned was clearly not derived from the mother, a homozygous yellow, but from the Japanese father.

Accordingly it was to be expected that the young would be heterozygous for the Japanese character, since they inherited the character from one parent only. Such proved indeed to be the case. An  $F_2$  generation, obtained by mating the Japanese young one with another, contained

11 Japanese and 7 yellow young, the most probable expectation being 13.5 Japanese to 4.5 yellow. Also when  $F_1$  Japanese individuals were mated with yellows, there were produced 117 Japanese and 128 yellow young. Evidently this is a 1 : 1 ratio. The slight excess of yellow young may have been due to a failure to classify as Japanese certain of the young which had very few black hairs in their coats and which may erroneously have been recorded as yellow or, as we shall see, it may be that a few of the Japanese young really did not have *any* black hairs in their coats. In no case did one of the Japanese young of either the  $F_2$  or the back-cross generation bear gray hairs. The hairs that contained black pigment were invariably black throughout their length.

When an  $F_1$  individual, heterozygous for the agouti factor as well as for Japanese, was mated with non-agouti yellow individuals, there were obtained both agouti and non-agouti yellows, and also agouti and non-agouti Japanese. At first it was found difficult to recognize the non-agouti Japanese, but after two individuals had been proved to be non-agouti by breeding tests in which they were mated to non-agouti yellows and produced only non-agouti yellow and Japanese young, no further difficulty was encountered. The under-surface of the tail, the belly, and the area around the eye are the best criteria, when they happen to be free from the Japanese mottling. In some of the most recently recorded litters, six non-agouti Japanese young were noted along with eight agouti Japanese young, the expected 1 : 1 ratio.

An  $F_1$  Japanese female was mated with a non-agouti black male heterozygous for yellow, producing three Japanese, one yellow and two black young, the expectation being 1 Japanese : 1 yellow : 2 black or gray. Another  $F_1$  Japanese female was mated with a blue angora male (with dominant extension factor) producing six black or steel gray young. One of these showed a lighter patch on one shoulder and was suspected accordingly of being heterozygous for the Japanese factor. When mated with yellow males she has produced 19 black young, 14 Japanese, and 4 yellow. The production of yellow young in this mating is contrary to expectation, since only black young and Japanese young should result, and these in equal proportions, if black, Japanese, and yellow are triple allelomorphs. Doubt might exist whether the yellow young are genetically as well as somatically yellow, for two of the Japanese young were at first recorded as yellow and so would have remained in the record had it not been discovered a few days later that each bore a few black hairs in its tail. Subsequent breeding tests made with these two animals proved them to be genetically Japanese-yellow heterozygotes. Three of

those recorded as yellow were destroyed before it was realized that the existence of such a class of young in this mating was problematical. The fourth individual, though it appears to be yellow, no black hairs having been detected anywhere on its body even after repeated careful inspections, is now adult and has been mated to a yellow female unrelated to the Japanese race, and has produced in two litters totalling 15 young, 5 clearly Japanese, the others being yellow. It is obvious therefore that this yellow animal is genetically a heterozygous Japanese. It seems probable therefore that such also were the other yellow young produced by this mother. She evidently is a heterozygote between black and Japanese and produces only two kinds of gametes, black and Japanese. In other words Japanese is a third allelomorph of black and of yellow, precisely like tortoise-shell in guinea-pigs. Since there are in rabbits two kinds of black, a dominant and a recessive kind, Japanese is really a *fourth* allelomorph of the extension factor. In order of dominance the four are  $E'$  (dominant black);  $E$  (ordinary recessive black);  $e^J$ , Japanese; and  $e$ , yellow.



# ON THE "JAPANESE" RABBIT.

By R. C. PUNNETT, F.R.S.

## INTRODUCTION.

SINCE 1917 I have been observing the behaviour of the so-called "Japanese" rabbit in crosses with various forms. Early in the present year (1924) I learned from Professor Castle that he also had been studying the heredity of the Japanese pattern during the past few years. As our experiments have led us independently to practically the same conclusion, I have adopted Professor Castle's suggestion that the two sets of records should appear in the same number of this *Journal*. Where references are made to Professor Castle's work in the following account they refer to the paper which immediately precedes the present one.

## THE JAPANESE RABBIT.

Except that it certainly has nothing to do with Japan little seems to be known of the origin of the Japanese rabbit. According to A. Wulf<sup>1</sup> it originated in France in 1887, and was subsequently improved in England. I have, however, seen animals of this pattern, along with other domesticated rabbits, exposed for sale in butchers' shops near Genoa, and should not be surprised if it were found to be generally, though sparsely, distributed among the mongrel rabbit population of Europe.

The only notes relating to the genetics of the Japanese rabbit would appear to be those of Endre Pap<sup>2</sup>, who gives data to shew that the pattern behaves as a simple recessive to full black. He also quotes Hagedoorn to the effect that the Japanese pattern is to be found in the blue and in the chocolate series as well as in the black. With this statement my own observations are in agreement, since both chocolate Japanese and blue Japanese have been produced in the course of my work. Pap also states that most Japanese rabbits shew some white Dutch markings<sup>3</sup>, thereby becoming tricolors, though there does not appear to be any linkage between the Dutch and the Japanese patterns. With this statement also my own work agrees. Tricolors appeared in  $F_2$  from a cross between a

<sup>1</sup> *Album der Rasse-Kaninchenzucht*, Würzburg, n.d.

<sup>2</sup> *Zeit. f. ind. Abst. Vererb.* Vol. xxvi. 1921.

<sup>3</sup> This of course is not true of the English breed.



Japanese and a "Spotted Dutch"<sup>1</sup> rabbit; nor did I observe any indication of linkage between the two patterns.

The Japanese rabbit exhibits very great variation in the amount of the dark marking, as well as in its distribution. The dark hairs may extend over the greater part of the body, though more diffused among the yellow in some regions than in others; or they may be represented by a few hairs only, so that the animal appears as a practically yellow rabbit. For the most part, however, the range of dark marking lies between these two extremes. A peculiar feature is the manner in which it tends to form transverse markings over the hinder part of the body. The ground colour of the belly is normally white, or nearly so; but it often appears greyish, owing to scattered dark hairs. In no region of the body are the dark hairs banded with yellow, even though the animal carries the agouti factor. Considerable variation in shade is to be found in the yellow ground colour of the coat, which may be anything between a clear creamy yellow, and a rich deep colour approaching that found in the so-called "New Zealand Red"<sup>2</sup>. I have not made any attempt to analyse the variations observed.

There is one further point of interest in connection with the Japanese pattern. An animal may carry the agouti factor, or it may carry the tan factor, or it may carry neither of these: i.e. when crossed with normal black a Japanese may give agoutis, or black and tans, or blacks, according to its genetical constitution. I have, however, failed to find any point of visible difference between these various Japanese. The belly may be as light in an animal that carries neither agouti nor tan as in an animal that does carry one or other of these two factors. Nor do the "tan" points of an agouti or a tan rabbit (i.e. the yellow marks on the feet, ears, nose, and nape of neck) serve to distinguish Japanese which carry the agouti or tan factors from those which do not. They may be obscured, or partially so, by black hairs, equally in a Japanese that contains the agouti or tan factors, and in one that does not.

In one case however the appearance of the Japanese would seem to serve as a guide to its genetical constitution. For in a Japanese which throws tortoise, and at the same time lacks the agouti factor, the general colour is more sooty, with yellowish belly and darker points as in a tortoise.

<sup>1</sup> For an explanation of this term cf. *Journ. Genetics*, Vol. ix. p. 309.

<sup>2</sup> This statement is meant to include all of the Japanese I have bred, the majority of which have been animals extracted from various crosses. In the pure bred Japanese the yellow ground colour is more uniform.

## EXPERIMENTAL DATA.

**A.** The original Japanese ♂ (*Q* 70)<sup>1</sup> was mated with a blue ♀ (*Q* 13) extracted from a Flemish-Polish cross, and known not to contain the factor which presents the appearance of agouti markings (= the *D*-factor of my earlier paper<sup>2</sup>, and the dominant extension factor of Castle). She produced eleven full blacks in two litters. Three *F*<sub>2</sub> litters from such *F*<sub>1</sub> blacks resulted in six blacks, five blues, four normal Japanese, and one blue Japanese.

**B.** The same Japanese ♂ (*Q* 70) was also mated with a chocolate ♀ (*Q* 111) shewing the "Spotted Dutch" type of marking. The four *F*<sub>1</sub> animals were black. From them was bred an *F*<sub>2</sub> generation consisting of fourteen black, six chocolate, five normal (= black) Japanese, three chocolate Japanese. Two of the black Japanese were of the tricolor type alluded to above on p. 231.

These matings served to shew that the Japanese pattern behaves as a simple recessive to self-colour, and that the black of the normal Japanese may be replaced by blue or by chocolate.

**C.** An unlooked for result was obtained when two of the black *F*<sub>1</sub> animals from **A** were mated back to Japanese. By this time the original pair of purchased Japanese had been disposed of. But a litter of five Japanese had been bred from them, and it was with two of these that the two *F*<sub>1</sub> blacks were mated. The results were:

*F*<sub>1</sub> ♂ (*Q* 144) × ♀ Jap. (*Q* 130) gave 2 agoutis, 2 blacks, 1 Japanese.

*F*<sub>1</sub> ♀ (*Q* 147) × ♂ Jap. (*Q* 129) gave 1 agouti, 4 blacks, 8 Japanese.

Since the *F*<sub>1</sub> animals did not carry the agouti factor, it must have been brought in by the Japanese (*Q* 129 and *Q* 130), and they must have received it from their mother (*Q* 71); for their father (*Q* 70) had already been shewn not to contain it. This meant that the individuals in a strain of Japanese breeding true to the characters of the breed, might nevertheless differ from one another in the presence or absence of the agouti factor. The presence of the agouti factor does not bring about banding in the black hairs of the Japanese. As earlier work had proved the existence of a black which is dominant to agouti, it was natural to regard the black of the Japanese as of this nature.

There was, however, a difficulty. The heterozygote *ex* dominant black × agouti is not black, but agouti-black, i.e. predominantly black but with some agouti banding<sup>3</sup>. Hence one would have expected in

<sup>1</sup> Purchased from the well-known breeder, Mr C. J. Davies.

<sup>2</sup> *Journ. Genetics*, Vol. II. p. 227, 1912.

<sup>3</sup> *Journ. Genetics*, Vol. II. p. 225, 1912, and Pl. XII. fig. 2.

the above experiment, not agoutis, but agouti-blacks. More recently, however, Onslow shewed that in some cases the heterozygous form between dominant black and agouti may be much nearer to agouti in appearance, taking the form of steel<sup>1</sup>. This suggested that the Japanese might be homozygous for a dominant black of which the heterozygous state, on an agouti basis, was not far removed in appearance from a rather dark agouti.

At this stage the Japanese experiments were dropped for a year owing to lack of accommodation, and it was not until the autumn of 1920 that I was able to return to them. A fact which called for explanation in Exp. C was that although both agoutis and blacks appeared among the self-coloured rabbits, yet all of the nine Japanese bred had the normal black markings. The non-appearance of animals with the Japanese pattern, but with agouti-banded hairs in place of black ones, suggested that the pattern and the dominant black were in some way linked together, and the following experiments were made.

**D.** A cross was made between the Japanese and the black Flemish, a form of black recessive to agouti. Some of the  $F_1$  animals were blacks and some were agoutis, the Japanese used being evidently heterozygous for agouti. Of the blacks 3 ♀♀ were mated to their brother, and the  $F_2$  generation consisted of 38 blacks and 10 Japanese, evidently a simple Mendelian relation. The absence of a tortoise class, as in Exp. A above, suggested that the relation between normal black, Japanese, and tortoise might be that of a series of multiple allelomorphs.

The agouti  $F_1$  animals (3 ♀♀ and 2 ♂♂) were also bred together, and in  $F_2$  produced 19 agouti, 6 black, and 9 Japanese, a close approach to a 9 : 3 : 4 ratio. All of the Japanese had the normal black markings, and again there was an absence of yellow and of tortoise.

A fresh point was brought out by this experiment. One of the  $F_1$  ♂♂ (S 27) shewed some dark hairs on the belly, while in the other (S 33) the left side of the head was much darker than the right, being in fact what might be described as dark steel. Some of the  $F_2$  agoutis were also noticed as being unusually dark. These facts suggested that the agouti which carries Japanese may have something of the Japanese pattern superimposed, as it were, upon the normal agouti type, and this has been confirmed by later observations. Japanese markings on an agouti are most easily determined by inspection of the belly of the baby rabbit of 1-2 weeks. At this stage the hair is short, and the dark hairs,

<sup>1</sup> *Journ. Genetics*, Vol. XII. 1922. Mr Onslow's results were known to me some time before he published them.

if present, are conspicuous. Later on when, as often happens, a darker underfluff appears, the Japanese markings tend to become lost unless they occur in large and well-marked patches. Elsewhere on the body the Japanese markings are rarely prominent; for being steel instead of full black, and generally diffuse, they grade into the rest of the coat. Moreover if the dark markings are scanty, as frequently happens in the Japanese, an agouti rabbit which bore them would not be distinguishable from an ordinary agouti. The densest black marks on an ordinary Japanese are those which may occur on the head, and it is on the head of the agouti which carries Japanese that one finds the most conspicuous Japanese markings, as in ♂ *S* 33 mentioned above.

**E.** Some results closely comparable with the above were obtained from the mating between a Japanese ♀ (*S* 4, known not to carry the agouti factor) and a homozygous black and tan ♂ for which I am indebted to my friend Mr T. H. Riches. The litter comprised seven black and tans, and these when bred together gave an  $F_2$  generation consisting of 35 black and tan, 14 black, and 16 Japanese—obviously a 9 : 3 : 4 ratio. Two of the Japanese were subsequently tested and shewn to be heterozygous for the tan factor. It was found that some of the  $F_2$  tans shewed irregular dark markings on the belly, while others were without such markings. Presumably the former belonged to the class of tan which carries Japanese.

The earlier experiments suggested that the factors for self-colour, Japanese, and dilute colour (yellow or tortoise) behaved as a series of multiple allelomorphs. For neither tortoise, nor yellow, nor tortoise tan appeared in the  $F_2$  generation from crosses between Japanese with black, agouti, or black and tan respectively. Hence dilute  $\times$  Japanese should give either dilute or Japanese in  $F_1$ , and a 3 : 1 ratio of the two classes in  $F_2$ .

**F.** Accordingly Japanese was mated with yellow, with the result that the seven  $F_1$  animals obtained were all Japanese. All of these animals, viz. five ♀♀ and two ♂♂ were used in the production of the  $F_2$  generation shewn in Table I.

It will be noticed that tortoise young as well as yellows were produced from ♀ *S* 41  $\times$  ♂ *S* 24. It was subsequently shewn by crossing with the black and tan ♂ (*S* 50) referred to above, as well as with a pure chocolate ♂ (*S* 81), that three of the ♀♀, viz. *S* 23, *S* 25, and *S* 46 were homozygous for the agouti factor, while the other two, viz. ♀ *S* 40 and ♀ *S* 41 were

TABLE I.

		Jap.	Yellow	Tortoise		Agouti	Tan	Black
♀ S 23	× ♂ S 24	31	18	—	× ♂ S 50	16	—	—
♀ S 25	× „	25	6	—	× „	10	—	—
♀ S 46	× „	10	3	—	× „	13	—	—
„	× ♂ S 42	9	6	—				
♀ S 40	× „	7	5	—	× „	2	2	—
„	× ♂ S 24	6	—	—	× ♂ S 81	2	—	5
♀ S 41	× „	13	1	2	× „	4	—	3
„	× ♂ S 42	4	3	—	× ♂ S 50	3	2	—
Total		105	42	2				

heterozygous (cf. Table I). Of the two ♂♂ one, S 24, was shewn to be heterozygous, for when mated with a chocolate ♀ (S 80) he gave four agoutis and threenon-agoutis: the other, S 42, was not tested on this point.

The  $F_2$  generation consisted of 149 animals, of which 105 were Japanese, and 44 yellows (including tortoise), expectation of course being 3 : 1. I am inclined to attribute the slight excess of yellows to the fact that the Japanese markings are sometimes so reduced that the animal may be mistaken for a yellow. This is supported by the fact that it is the earlier litters that are responsible for the excess of yellows. On dividing the  $F_2$  population into two halves consisting of the earlier and the later litters respectively I find that the former contains 47 Japanese : 27 yellow, and the latter 58 Japanese : 17 yellow. At the time the earlier litters were recorded I had not appreciated the extreme reduction which the Japanese pattern might sometimes undergo, and it is not improbable that a few of the young recorded as yellows were genetically Japanese.

Of the agoutis produced by crossing ♀ S 23 and ♀ S 46 with the black and tan ♂ S 50, a buck and several does were mated together. Independent tests had shewn that some of these agouti ♀♀ were heterozygous for yellow, and some for Japanese (others remaining yet untested), while the buck carried Japanese. The matings might therefore be expected to give agoutis, tans, and Japanese only, in the ratio 9 : 3 : 4. The actual numbers, 46 agouti, 10 tans and 18 Japanese are not far removed from the expected ratio. Of both the tans and the agoutis some were normal, while others shewed Japanese markings on the belly. The proportions of the Japanese marked agoutis and tans would differ according as the does used carried Japanese or yellow. If they carried the former the Japanese marked agoutis and tans should be twice as numerous as the normal ones<sup>1</sup>: if the latter, the normals should be twice

<sup>1</sup> Assuming the markings always to shew in animals carrying Japanese. In cases where the Japanese marking is much reduced these dark-coloured animals would almost certainly not shew it.

as numerous as the Japanese marked ones. This point cannot, however, be dealt with further, since the constitution of all of the nine does used has not yet been determined.

G. Lastly I may make mention of a cross between Japanese ♀ and tortoise ♂. The seven  $F_1$  animals were from their appearance termed "Tort-Japs," for in general colour they were tortoise, though with some Japanese markings. In all of them however the markings were slight, in two cases being reduced to a few black hairs on the back. A small  $F_2$  generation was bred and recorded as 9 Japanese, 3 yellows, and 11 tortoise. Some of the Japanese were recorded as normal, and others as "Tort-Japs." In almost all of them the markings were scanty, and I am inclined to consider that the three animals recorded as "yellow" were genetically Japanese, in which the black markings were almost absent. It is also possible that some of those recorded as tortoise may have been "Tort-Japs."

#### DISCUSSION OF DATA

The various experiments recorded above are clearly in accordance with the view that normal black, tortoise and Japanese are alternative in heredity. And since we know that D-black behaves as though it were alternative to normal black, we should naturally expect it to fall into the same series. This expectation has been translated into fulfilment by Professor Castle's work. We are dealing therefore with a series of four allelomorphs corresponding to the four alternative characters:

Dominant black	...	D
Japanese	...	d
Recessive black	...	E
Tortoise	...	e

And this would appear to be as far as purely Mendelian analysis can at present take us.

Yet, though we leave firm ground, a few speculations may not be out of place. We have seen that the black of the Japanese behaves as though it were of the nature of the D-black. May we not regard the Japanese as standing in something of the same relation to D-black as tortoise does to E-black<sup>1</sup>? There is in either case a diminution of melanic pigment with a corresponding increase in yellow. In neither case is the

<sup>1</sup> The relation is unlikely to be the same. For if so E-black × Japanese should give rise to some tortoise animals in  $F_2$ . If we take the view that we are dealing with an intensity-dilution pair common to both kinds of black we must make the further supposition that there is very close linkage between such a distributional pair and the two blacks, a supposition for which at present there is no experimental evidence.

dilution uniform all over the body. The black pigment in the tortoise is denser in certain regions, viz. nose, ears, feet, and tail; and in some tortoise it is far denser than in others. In the Japanese the areas of density are far less regular, though the black pigment tends to collect in certain regions of the body more than in others. When reduced in amount it tends to disappear, roughly, in those regions of the body where a Dutch marked rabbit is white<sup>1</sup>. But although the melanic pigment tends to disappear it never does so completely, for even in the most yellow areas of a Japanese rabbit careful search will reveal the existence of occasional dark hairs. Melanic pigment is to be found all over the body of the Japanese, but, in its dilute form, the D-black pigment does not "spread" well, and so we obtain an apparently mosaic effect. The dilute E-black spreads more evenly, and though there is a greater density of black in the "points," as in a Himalayan, the darker areas grade uniformly into the lighter. But the light hairs, unlike those of the Japanese, contain some melanic pigment.

The apparent identity of Japanese black with D-black is brought out in an interesting way in the relation of them both to the agouti factor. In a Japanese containing the agouti factor (A) the black hairs, as we have seen, remain unbanded. And this is equally true whether the animal be homozygous or heterozygous for d. For the animal represented by the formula ddAA is visibly indistinguishable from that represented by deAA. Some years ago I drew attention to the fact that, where all carried the agouti factor, the homozygous dominant black was indistinguishable in appearance from the dominant black which carried yellow<sup>2</sup>. Adopting our present set of symbols both DeAA and deAA rabbits are full blacks, without any trace of agouti banding. The agouti factor fails to lead to banding where the factor for dominant black, whether in the intense (D) or in the dilute (d) state, is on a basis of yellow, i.e. in the absence of the E factor. When however the E factor is present in addition to the factor for agouti, the black produced by the dominant black factor, whether in the intense or the dilute state, is subject to banding. The DEAA animal shews some agouti banding. The corresponding animal in the dilute form, the dEAA animal, is, visibly, a Japanese agouti. The yellow area of the Japanese becomes agouti, while the black areas become dark steel or agouti-black.

<sup>1</sup> I have occasionally bred Japanese which might not unfairly be described as irregularly Dutch marked, the white being replaced by yellow, though the dark areas were, of course not fully black.

<sup>2</sup> *Journ. Genetics*, Vol. v. 1915, p. 47.

If we regard Japanese as a dilute form of dominant black we must look upon yellow as a basis underlying these four forms of black, viz. D-black, d-black (= Japanese), E-black, and e-black (= tortoise). We do not at present know of a yellow without melanic pigment, and must consider the four members of the allelomorphic series under discussion as corresponding to four states of melanic pigmentation. Yellow must be supposed to underlie the two intenser forms of black as well as the two dilute ones. This view is in accordance with the result of crossing E-black with chinchilla, an agouti which lacks yellow. That such a cross gives normal agoutis supports the view that the E-black carries yellow. But here, and probably also in the D-black, the yellow is crowded out, as it were, by the great development of the melanic pigment.

However, we are still faced with the problem of framing some conception of the biochemical relation that obtains between our two forms of black—the D-black and the E-black. Either we must regard them as chemically distinct, or we must suppose that they are chemically similar, and that the differences in their genetical behaviour are due to some difference in the respective mediums in which they are produced. That the latter possibility is the less probable seems to be indicated by the existence of the Japanese-agouti, which is best interpreted by supposing that the E-black is uniformly distributed, while at the same time the D-black is distributed irregularly. The fact that this takes place in the same animal certainly suggests that we are concerned with two distinct pigments. And this is supported by the further fact that the "Tort-Jap" shews both the black patches of the Japanese and the dilute melanic pigmentation of the tortoise. The case would be an interesting one for biochemical investigation.

*Note.* Since the above account was written my friend, Mr J. B. S. Haldane, has kindly sent me the following data from experiments made by himself and L. K. Haldane. The Japanese used in this work was bred from my original stock.

1. From the cross Japanese  $\times$  yellow, or Japanese  $\times$  tortoiseshell, an  $F_2$  generation was raised consisting of 66 Japanese and 21 yellow or tortoise.

2.  $F_1$  ex Japanese  $\times$  tortoise, or  $F_1$  ex Japanese  $\times$  yellow, mated back either to yellow or tortoise, gave 26 Japanese and 27 yellow or tortoise.

These results are closely in accordance with the simple Mendelian



relation between Japanese on the one hand, and yellow or tortoise on the other already suggested by Professor Castle and myself.

3. An  $F_2$  Japanese buck from 1, with only a small patch of black on one cheek and on one flank, was mated with several yellow and tortoise does. He gave 8 yellow or tortoise and 11 Japanese, all of which latter were more heavily marked with black pigment than himself. The case is of interest in view of Professor Castle's statement (p. 228 above) that a rabbit, which is genetically a Japanese-yellow heterozygote, may be without the black markings characteristic of the Japanese.

# STUDIES ON THE INHERITANCE OF THE WEIGHT OF NEW-BORN RABBITS.

BY STEFAN KOPEĆ.

*Government Institute for Agricultural Research, Pulawy, Poland.*

(With two text-figures.)

## CONTENTS.

	PAGE
Introduction . . . . .	241
Method of investigation . . . . .	242
A. The Himalayan ♀ × Silver ♂ cross . . . . .	244
The $F_1$ generation . . . . .	244
The $F_2$ generation . . . . .	246
The $F_3$ generation . . . . .	250
B. The Silver ♀ × Himalayan ♂ cross . . . . .	252
The $F_1$ generation . . . . .	252
The $F_2$ generation . . . . .	253
Conclusions and summary . . . . .	254
Literature referred to . . . . .	256
Tables of data . . . . .	258

## INTRODUCTION.

PRELIMINARY investigations on the inheritance of body-weight in rabbits are given by Castle (1) *et al.*, who, in their experiments, arrived at the conclusion that full-grown specimens of  $F_1$  are of intermediate weight, and that this character does not undergo segregation. The intermediate weight of the  $F_1$  generation in rabbits was confirmed by MacDowell (22). This author observed, however, that the variability of weight in full-grown individuals is in general smaller in  $F_1$  than in back-cross fraternities. MacDowell infers therefrom that the weight of rabbits is inherited in accordance with Mendel's law, and by the interaction of multiple factors. The recent, more detailed researches of Castle (3) are in agreement with this opinion. On the other hand, partly different results were arrived at by other authors. A shifting of body-weight in  $F_1$  generation in the direction of the lighter breed has been noticed by Davies (4). The same has been ascertained by Punnett and Bailey (24) in full-grown  $F_2$  rabbits. We see that the investigations on the inheritance of body-weight in rabbits yield no concordant results. This may be

caused by difficulties met with during accumulation of sufficiently large experimental materials of full-grown rabbits. I therefore resolved to rely in my research on the weight of new-borns. Although I do not suppose that the mode of inheritance of body-weight at birth is different from that of adult rabbits, I nevertheless want to remark that I have no intention to extend here to adult animals the results obtained on new-borns. The validity of such generalisation can only be ascertained by means of further methodical experiments. It has been emphasised by Castle (3) that the heavier new-born may in time become lighter than those which had been born lighter. But it ought to be said that such a possibility does in no way solve the question as to the absence of an essential positive correlation between the weight of new-born and that of fully-developed animals. On the contrary, the observations made by Dunn (5) and King (16) point to the existence of such correlation in albino rats, at least in so far as very small new-born are concerned. The data recorded by Eckles and Swett (7) speak to a certain degree in favour of an analogous connection between the weight of calves at birth and the mature height at withers.

#### METHOD OF INVESTIGATION.

The rabbits were weighed at birth always before their first suckling (cf. Kopeć (20)). Fractions of grams were considered as full grams. Since weight of the new-born varies inversely with the number of specimens in the litter (Kopeć (20), Hammond (9)), the present data refer only to such litters as are most frequent, *i.e.* those containing four to six rabbits. In this manner 825 new-born were examined (cf. Table 8). It ought to be remarked that I have not been able to discriminate the sex of the new-born, as the method of Jackson (13), referring to new-born rats, failed in rabbits. But as the weight-curves of separate populations have never been bimodal, I do not suppose the weight of the two sexes to differ essentially at birth. Biometrical constants were, as a rule, calculated from the usual formulae. (On account of the small number of specimens in separate materials a special formula has been used for the standard deviation, viz.  $\sigma = \pm \sqrt{\frac{\sum (pa)^2}{n-1}}$   $a$  being the difference between single variates and the mean,  $p$  the number of the variates and  $n$  the total number of specimens examined.)

Owing to restricted means, I only made crosses of Himalayan with Silver rabbits. The greatest weight attained by full-grown Himalayan

does from my breed during the first two years of their life was from 1675 to 2015, on the average 1831 grams. The corresponding weights of Silver females were from 1991 to 2518, on the average 2286 grams. The breeds employed for the crosses differed therefore from each other only to a small degree. But as may readily be inferred from Tables 1, 2 and 8, the new-born of "pure" Himalayans are, on the average, lighter than those of Silver rabbits, and consequently these breeds are suitable for investigations on the inheritance of the weight of new-born.

In previous investigations only one pair of parents has usually been selected as starting material, and we never are able to decide whether or not the large or the small weight of a specimen constitutes an essential character dependent on a specific genotype of the individual under examination. Such research ought to be based on averages representing the essential value (in a biometrical sense) of the weight of the examined breeds, irrespective of their variability. This is especially important in cases where the breeds used for the cross differ but slightly in their respective weights. The same standpoint has been lately accepted by Castle (3), who, however, owing to very scanty number of *P* animals, considers his own researches on the variability of body-weight in full-grown rabbits as only partially successful. By using as material the new-born, which are more easy to procure, I was able to obtain more exact curves of variability, and therefore more fit for biometrical examination.

The methods of research hitherto employed on full-grown rabbits have always involved loss of numerous individuals which died before having attained the age appointed for observation. This circumstance is taken into consideration by Castle (3), who endeavoured to check the loss of smaller individuals by using foster-mothers. The mortality among his animals was nevertheless considerable. The same is undoubtedly true in regard to the experiments of other authors. Castle remarks, it is true, that thorough examination of the dead specimens does not reveal any evidence of selective mortality, but in this respect also my method may be better relied upon, as dealing with total offspring of separate females.

Stress must also be laid on the fact that the weight of developing animals is influenced by external conditions. My method of examining the weight of the new-born involves only such irregularities as are caused by the action of the developmental conditions in the maternal body, which constitute the "external" conditions of the growing foetus. On the other hand, with full-grown animals we must take into account the more complex conditions of post-foetal life. It may be, it is true, supposed that unequal external conditions of the postnatal life in certain

cases neutralise the differences between separate individuals which were caused in the uterus, but in other cases differences between the phenotype and the biotype of the examined populations may be further increased by these conditions. Important observations in this respect were made by Jackson (13), who ascertained that the coefficient of variability of weight in albino rats is smallest at birth.

Lastly, I may mention that in their experiments on fully-developed rabbits the authors cited take their data from animals born at different periods of the year. Even if the animals were fed identically, the period of life during which they received green summer food (which is of known importance for growth), fell, in different specimens, in different stages of their respective development. My method allowed me to restrict myself to the examination of females during several summer months, when the food administered to the animals could be alike. In these conditions the influence of variable food quality on the weight of the new-born was reduced to a minimum.

#### A. THE HIMALAYAN ♀ × SILVER ♂ CROSS.

##### *The $F_1$ generation.*

In these crosses seven Himalayan females were used, viz. Nos. 8, 10, 11, 25, 36, 77 and 118. They were mated to the Himalayan male No. 7, in order to establish the weight of their "pure-bred" offspring (Table 1), as well as to Silver buck No. 44, belonging, as mentioned, to a heavier breed (Table 3). Since females Nos. 36 and 77 were daughters of female No. 8 and male No. 7, and female 118 of female No. 11 by the same male, we are concerned here with inbreeding. The figures show that the inbreeding had no negative influence on the weight of new-born. On the contrary, we may infer from the data of Tables 1 and 9 that the weight of the inbred new-born was in general even larger than that of those not inbred<sup>1</sup>.

On comparing the data from Tables 1 and 3 we see that the weight of separate new-born of the  $F_1$  generation from this cross is larger than that of pure-bred Himalayans produced by the same females. The same may be inferred from the average weights evidenced in Table 9. Biometrical estimation of a difference is based on the ratio of this difference

<sup>1</sup> These observations are concordant with those made by Huth (12) on the influence of inbreeding on full-grown rabbits, being in disagreement with the results obtained by Punnett and Bailey (24), who ascertained a distinct negative influence of inbreeding on the weight of fully-developed specimens. Conformably with Punnett and Bailey's opinion referring to the above mentioned results by Huth, as well as to the well-known investigations of King (17) in inbred albino rats, the increase of inbred new-born in my material may possibly be considered as due to chance selection of the does used to start with.

to its probable error, only those differences being considered as significant which are approximately equal to or greater than four times the probable error ( $\frac{\text{Diff.}}{\text{E. Diff.}} \geq 4$ ). On surveying the items of Table A, calculated from corresponding data of Table 9, we remark that in all cases the differences between the average weights of the  $F_1$  generation and of those of

TABLE A.

*Differences of the average weights and of the coefficients of variability of weight between the  $F_1$  offspring from Himalayan ♀ × Silver ♂ cross and the pure-bred Himalayan new-born.*

No. corresponding to each mother	Differences of the average weights		Differences of the coefficients of variability	
	Difference ± probable error	Difference : probable error	Difference ± probable error	Difference : probable error
8	9.32 ± 0.86	10.8	-4.45 ± 1.73	2.6
10	5.20 ± 0.85	6.1	-5.15 ± 1.51	3.4
11	4.97 ± 0.87	5.7	1.71 ± 1.78	1.0
25	6.25 ± 1.01	6.2	-1.96 ± 1.77	1.1
36	2.37 ± 1.21	2.0	-9.38 ± 2.23	4.2
77	2.53 ± 1.35	1.9	2.02 ± 2.25	0.9
118	17.62 ± 1.12	15.7	-4.78 ± 1.91	2.5

Himalayan new-born are positive, though rather low for the offspring from ♀ 36 and from ♀ 77.

If we consider the whole material of new-born (Table 8), we observe that the greatest number of variates is found in the class 30–35 in pure bred Himalayans, in the class 45–50 in Silver new-born, and in the  $F_1$  generation of the cross examined in the class 35–40 grams (cf. Text-figure 1). The average weight of Himalayans at birth is  $35.92 \pm 0.33$ , of Silver individuals  $44.19 \pm 0.34$ , and in the hybrids it has the intermediate value of  $41.91 \pm 0.37$  grams. The difference of the average weight of the  $F_1$  generation and that of Himalayans is  $5.99 \pm 0.50$ , the corresponding difference in relation to hybrids and Silvers being  $-2.28 \pm 0.50$  grams. In both cases the difference is significant, being larger than its fourfold probable error.

From the items of Table 9 it is seen that the coefficient of variability of the Himalayan offspring from the females studied is in two cases larger than that of hybrids from the same mothers (♀ 11 and ♀ 77); in the remaining cases, on the contrary, it is smaller. From Table A it may however be found that the differences of these coefficients are biometrically insignificant, with the exception only of the offspring of female No. 36, as in this case alone the relation of the difference to its probable error is sufficiently large, being 4.2. In other words, there is no

significant regular change with regard to variability of weight in the  $F_1$  generation as compared with that of pure Himalayan offspring. This is confirmed by the values of the standard deviations, *i.e.* indexes of variability in both materials (cf.  $\sigma$  in Table 9).

*The  $F_2$  generation.*

To facilitate reference, the notation adopted for  $F_1$  animals is such as to indicate the Himalayan doe from which they are derived; *e.g.* the daughter of ♀ 8 and ♂ 44 is denoted by ♀ 8<sub>1</sub>, the daughter of ♀ 11 by the same male as ♀ 11<sub>1</sub>, etc. Corresponding  $F_1$  males are denoted as ♂ 8<sub>1</sub>, ♂ 11<sub>1</sub>, etc.

The data of Table 9 show that the  $F_2$  generation from females Nos. 8<sub>1</sub>, 10<sub>1</sub>, 11<sub>1</sub> and 25<sub>1</sub> is on the average heavier than that of the  $F_1$  generation, while of the remaining three cases the opposite is true (cf. also the data of Table 4 with those from Table 3).

Table B (calculated from the data of Table 9) shows that the differences are only in three cases biometrically significant, being negative in

TABLE B.

*Differences of the average weights and of the coefficients of variability of weight between the  $F_2$  and the  $F_1$  offspring from Himalayan ♀ × Silver ♂ cross.*

No. corresponding to each mother	Differences of the average weights		Differences of the coefficients of variability	
	Difference ± probable error	Difference : probable error	Difference ± probable error	Difference : probable error
8 <sub>1</sub>	0.22 ± 1.08	0.2	9.93 ± 1.85	5.4
10 <sub>1</sub>	0.69 ± 1.10	0.6	8.46 ± 1.80	4.7
11 <sub>1</sub>	5.33 ± 1.25	4.3	10.76 ± 2.22	4.8
25 <sub>1</sub>	3.25 ± 1.40	2.3	7.91 ± 2.19	3.6
36 <sub>1</sub>	-5.05 ± 1.36	3.7	14.71 ± 2.70	5.4
77 <sub>1</sub>	-3.44 ± 1.30	2.6	-1.14 ± 2.18	0.5
118 <sub>1</sub>	-9.83 ± 1.15	8.5	8.21 ± 1.79	4.6

two cases, and in one case positive. The difference between the average weights of the total material of the  $F_2$  and of the  $F_1$  generation, calculated from the data of Table 8 is  $-0.85 \pm 0.52$ , and must therefore be considered as fortuitous (cf. Text-figure 1). Consequently the average weight of the  $F_2$  generation does not evidence any distinct change as compared with that of the  $F_1$  generation, *i.e.* the average weight of the first filial generation is maintained in the second generation.

Apart from this, we find in the  $F_2$  generation a considerably larger variability of new-born weight as compared with that of the  $F_1$  generation (cf. Tables 3 and 4). The increased variability of the  $F_2$  new-born may be ascertained biometrically. The data of Table 9 show that the

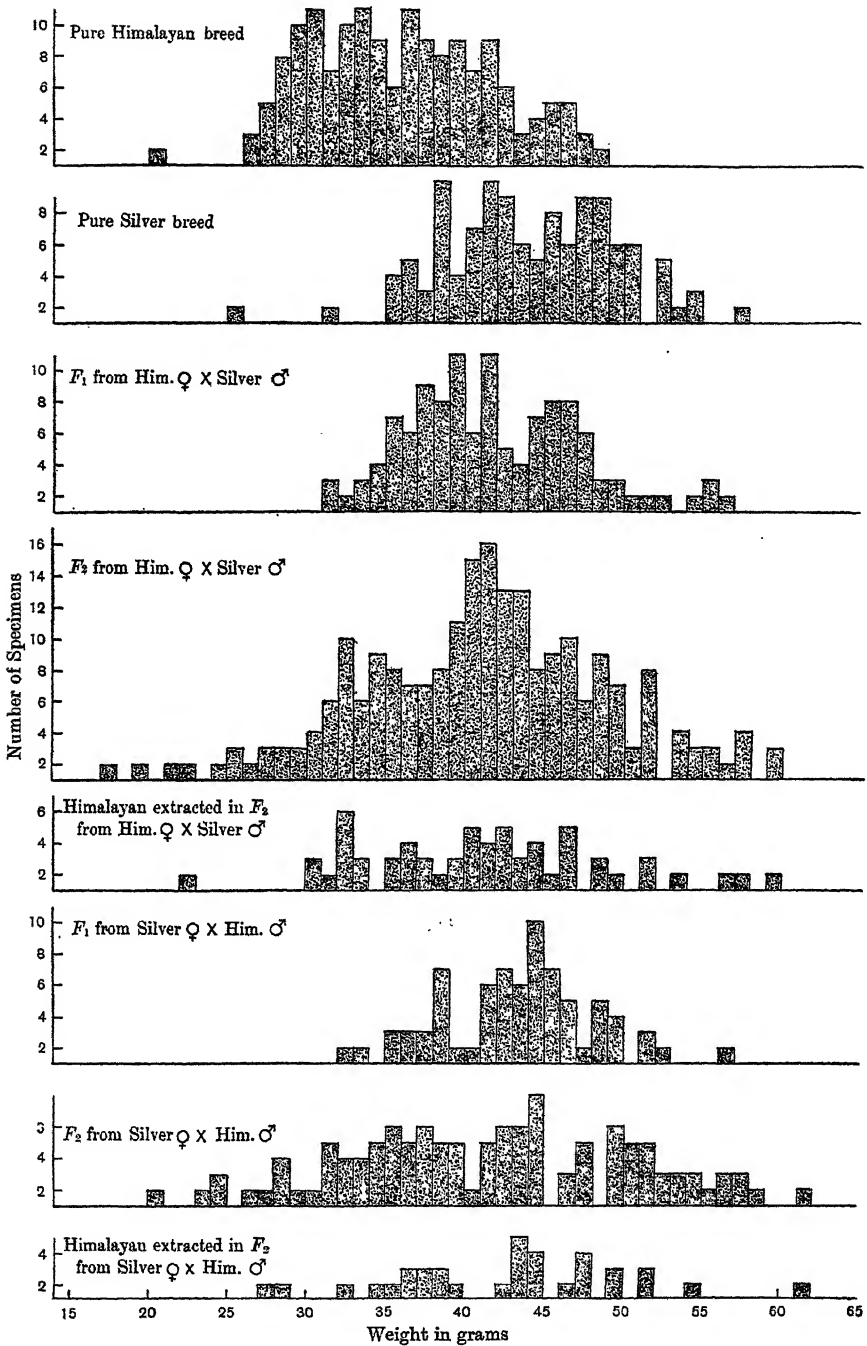


Fig. 1.



coefficients of variability are (with the only exception of the offspring from ♀ 77) much larger in  $F_2$  than in  $F_1$ . We see moreover from Table B that the difference of these coefficients in the exceptional case of the progeny of ♀ 77 is biometrically insignificant, in contrast to the remaining cases in which the differences between the respective coefficients in new-born of the  $F_2$  and of the  $F_1$  generation may be considered as significant. In connection with litter-size, which in general was larger in  $F_2$  than in  $F_1$  (cf. Tables 8 and 9), previous observations showed the number of specimens in one litter has no influence on variability of weight in new-born rabbits (Kopeč (19)).

Let us now take into consideration the relation of the weight of the Himalayan new-born extracted in  $F_2$  generation to that of the pure-bred Himalayans from the  $P$  generation (cf. Tables 4 and 1). It turned out that the Himalayan new-born extracted in the  $F_2$  generation are heavier than the pure-bred Himalayan new-born from the females of the  $P$  generation which were employed in the examined cross. The data of Table C show that only in one case (offspring of ♀ 36) rather a contrary behaviour may be noticed; however, the biometrical differences between

TABLE C.

*Biometrical constants referring to the Himalayan new-born extracted in  $F_2$  from the Himalayan ♀ × Silver ♂ cross.*

No. corresponding to each mother	Average weight and its probable error	$\pm \sigma$	Total number of specimens	Difference between the average weight of the extracted and the pure-bred Himalayan new-borns	
				Difference $\pm$ probable error	Difference: probable error
8 <sub>1</sub>	39.54 $\pm$ 1.59	8.55	13	7.59 $\pm$ 1.71	4.4
10 <sub>1</sub>	41.71 $\pm$ 1.14	4.46	7	4.12 $\pm$ 1.30	3.2
11 <sub>1</sub>	43.83 $\pm$ 2.47	8.95	6	12.13 $\pm$ 2.52	4.8
25 <sub>1</sub>	47.33 $\pm$ 1.60	5.82	6	10.50 $\pm$ 1.71	6.1
36 <sub>1</sub>	37.33 $\pm$ 0.90	3.27	6	-0.47 $\pm$ 1.43	0.4
77 <sub>1</sub>	42.13 $\pm$ 1.81	7.59	8	1.44 $\pm$ 1.96	0.7
118 <sub>1</sub>	42.80 $\pm$ 3.29	10.89	5	8.20 $\pm$ 3.37	2.4

the average weights of both Himalayan materials recorded in this table are significant in three cases only. But since the number of Himalayan new-born extracted in  $F_2$  was very scanty (amounting only to from 5 to 13 individuals) the items calculated for the progeny of each mother separately are scarcely adapted for biometrical examination on account of the large probable error. Clear results were arrived at only by comparing all pure-bred Himalayans with all Himalayan new-born extracted in  $F_2$ . From Table 8 we find that the greatest class-frequencies fall in the first case in the class 30–35, in the second lying in the class 40–45 grams

(cf. Text-figure 1). The difference between the general average weights established from the data of Table 8 amounts to  $5.50 \pm 0.76$ , the pure-bred Himalayans being lighter. The difference, being more than seven times its probable error, must be considered significant.

The question arises as to whether the Himalayan new-born extracted in the  $F_2$  generation show any difference of weight as compared with the remaining animals of the same generation, which was composed partly of extracted pure Silver rabbits and partly of hybrids similar to those from  $F_1$  generation, having, when full grown, the same uniformly black-silver pattern. Silver rabbits cannot be discriminated from hybrids in the new-born stage, both kinds having the same greyish-blue colour.

The extracted Himalayans could consequently be compared only with all non-Himalayan specimens produced by the same females without discriminating the Silver from the hybrid new-born (cf. items of Table 4). The average weight of the non-Himalayan new-born, calculated from the data of Table 4, are recorded in Table D. On surveying

TABLE D.

*Biometrical constants referring to the non-Himalayan new-born belonging to the  $F_2$  from the Himalayan ♀ × Silver ♂ cross.*

No. corresponding to each mother	Average weight and its probable error	$\pm \sigma$	Total number of specimens	Difference between the average weight of the non-Himalayan and Himalayan new-borns of $F_2$	
				Difference $\pm$ probable error	Difference: probable error
8 <sub>1</sub>	42.64 $\pm$ 1.12	7.77	22	3.10 $\pm$ 1.95	1.6
10 <sub>1</sub>	44.17 $\pm$ 1.22	7.67	18	2.46 $\pm$ 1.67	1.5
11 <sub>1</sub>	41.54 $\pm$ 1.13	8.19	24	-2.29 $\pm$ 2.72	0.8
25 <sub>1</sub>	46.00 $\pm$ 1.43	8.97	18	-1.33 $\pm$ 2.15	0.6
36 <sub>1</sub>	33.91 $\pm$ 1.90	9.33	11	-3.42 $\pm$ 2.10	1.6
77 <sub>1</sub>	39.11 $\pm$ 0.69	5.43	28	-3.02 $\pm$ 1.94	1.6
118 <sub>1</sub>	41.12 $\pm$ 0.73	5.55	26	-1.68 $\pm$ 3.37	0.5

the data of this table as compared with those recorded in Table C we see that in cases of the progeny of mothers 8<sub>1</sub> and 10<sub>1</sub> the Himalayan young were lighter, in the remaining cases they were even heavier, than the non-Himalayan progeny of the same does. From the data of Table D we may infer that the difference was in no case biometrically significant. The average weight of all non-Himalayan  $F_2$  rabbits calculated from items of Table 8 was  $40.95 \pm 0.44$ , the difference between this weight and the corresponding average weight of Himalayan young of the same generation was only  $-0.47 \pm 0.81$ , therefore of no significance. In respect of weight there is no difference between the Himalayan and the non-Himalayan components of the  $F_2$  generation.

*The  $F_3$  generation.*

I have, unfortunately, been able to make only very restricted investigations of the  $F_3$  generation (cf. Tables 5 and 10). The seven full-grown females used were daughters from ♀ 36<sub>1</sub> by her brother. Four

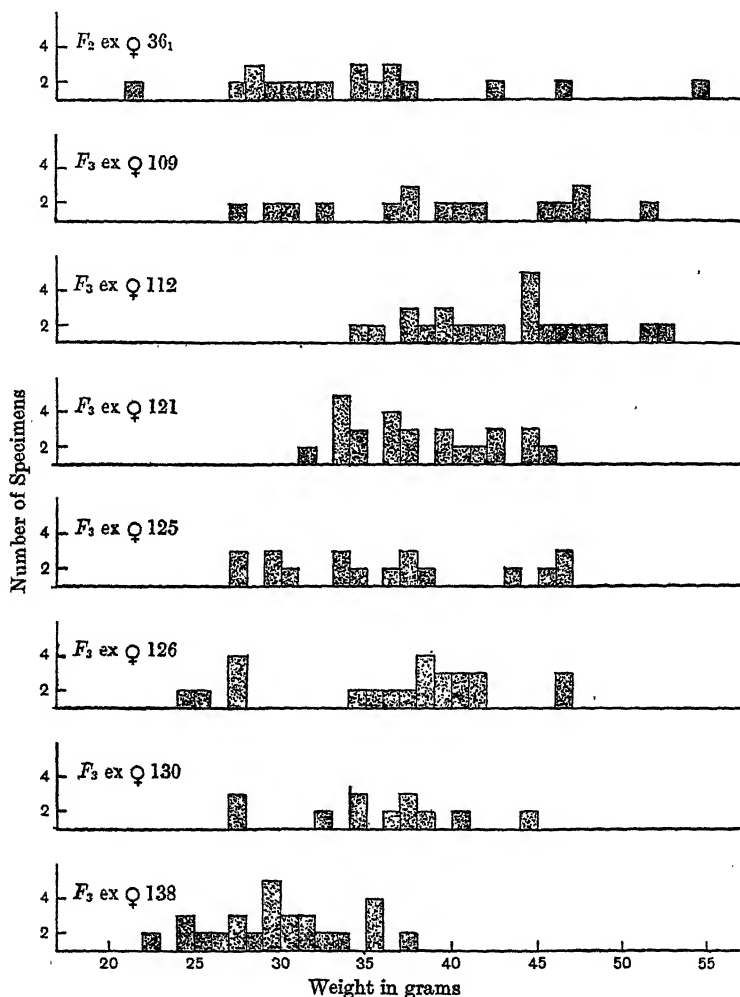


Fig. 2.

among them (Nos. 109, 121, 125, 126 and 138) were black-silver, the remaining two (Nos. 112 and 130) were extracted Himalayans. The first were mated to their dark-silver brother ♂ 128, the extracted Himalayans

to their Himalayan brother ♂ 129. By comparing the average weights of the new-borns of the  $F_3$  generation, from each female separately, with the corresponding weight of the  $F_2$  generation produced by ♀ 36<sub>1</sub> (which was  $35.12 \pm 1.27$ ), we find that the new-born weight in the  $F_3$  generation is in certain females distinctly different in relation to that of the  $F_2$  generation (cf. Table 10 and Text-figure 2). In one case (offspring of ♀ 112) this weight was biometrically larger in  $F_3$  than in  $F_2$ : the relation of the corresponding difference to its probable error is here as large as 5.6, and is therefore significant (cf. Table E). In the case of the offspring

TABLE E.

*Differences of the average weights and of the coefficients of variability of weight between the  $F_3$  and the  $F_2$  offspring from Himalayan ♀ × Silver ♂ cross.*

No. corresponding to each mother	Differences of the average weights		Differences of the coefficients of variability	
	Difference ± probable error	Difference : probable error	Difference ± probable error	Difference : probable error
109	$4.81 \pm 1.80$	2.7	$-3.82 \pm 3.42$	1.1
112	$8.23 \pm 1.48$	5.6	$-10.48 \pm 2.86$	3.7
121	$3.45 \pm 1.43$	2.4	$-10.59 \pm 2.84$	3.7
125	$1.51 \pm 1.69$	0.9	$-4.19 \pm 3.35$	1.3
126	$1.98 \pm 1.63$	1.2	$-3.99 \pm 3.22$	1.2
130	$0.97 \pm 1.64$	0.6	$-7.96 \pm 3.29$	2.4
138	$-4.67 \pm 1.40$	3.3	$-9.04 \pm 2.90$	3.0

of the mothers Nos. 109, 121, 125, 126 and 130, this weight is, it is true, apparently larger in  $F_3$ , but the corresponding difference exceeds its probable error 0.6 to 2.7 times only, and consequently the weight of the new-born from these five females ought to be considered as equal to that of the  $F_2$  generation. In the last case (offspring of female No. 138) the  $F_3$  generation was distinctly lighter than the corresponding  $F_2$  new-borns. The relation of the difference to its probable error was here 3.3, i.e. approximately the obligatory number four. It ought, however, to be noticed that the average number of young in a litter was, in the case of female No. 130, only 4.5, while the average number of  $F_2$  new-born produced by female 36<sub>1</sub> was 5.7. On the other hand, it has been pointed out that there exists a real inverse correlation between the litter-size and the weight of new-born rabbits. Hence follows that by using the appropriate regression we should find a still smaller average new-born weight in  $F_3$  from ♀ 138 as compared with the corresponding  $F_2$ . The relation of the difference between these weights to its probable error would more closely approach four. Hence I venture to maintain that the  $F_3$  generation from ♀ 138 was really lighter than the  $F_2$  generation. From the whole

## 252 *Inheritance of the Weight of New-born Rabbits*

of the above consideration it follows that the average weight of the new-born underwent a certain segregation in  $F_2$  (cf. Text-figure 2).

The variability of weight of new-born of the  $F_3$  generation underwent a remarkable change as compared with the  $F_2$  generation. On comparing the coefficients of variability of  $F_3$  new-born (Table 10) with the coefficients of variability of  $F_2$  new-borns from ♀ 36<sub>1</sub> (which was  $22.18 \pm 2.57$ ), we find that in all cases without exception these coefficients are smaller in  $F_3$ , being here only from 11.59 to 18.36. The differences between these coefficients and the coefficient  $22.18 \pm 2.57$  recorded in Table E have, however, a considerable probable error. In regard to this, although the remarkably regular decrease of variability of new-born weight in the  $F_3$  generation as compared with  $F_2$  specimens cannot be biometrically proved, it must be regarded as highly probable.

### B. THE SILVER ♀ × HIMALAYAN ♂ CROSS.

#### *The $F_1$ generation.*

This cross was carried out on Silver does. They were all mated with the same Silver buck No. 44, in order to obtain "pure" Silver new-born (Table 2), and with the male No. 7 belonging to the lighter Himalayan breed, with the view to producing the corresponding  $F_1$  generation of hybrids (Table 6). On surveying the data of Table 11 we find that in this reciprocal cross the intermediate character of the weight of  $F_1$  new-born cannot as readily be ascertained as in the offspring from the Himalayan ♀ × Silver ♂ cross. In the cases of the offspring of ♀ 5 and ♀ 6 the hybrid new-born were lighter than pure Silver young, while the opposite was true for the offspring of ♀ 29 and ♀ 92. From Table F

TABLE F.

*Differences of the average weights and of the coefficients of variability of weight between the  $F_1$  offspring from Silver ♀ × Himalayan ♂ cross and the pure-bred Silver new-born.*

No. corresponding to each mother	Differences of the average weights		Differences of the coefficients of variability	
	Difference ± probable error	Difference : probable error	Difference ± probable error	Difference : probable error
5	-3.63 ± 0.99	3.7	0.71 ± 1.56	0.5
6	-5.15 ± 0.82	6.3	2.79 ± 1.34	2.1
29	7.02 ± 1.09	6.5	-3.17 ± 1.74	1.8
92	4.25 ± 1.19	3.6	-2.51 ± 1.86	1.3

(plotted from the data of Table 11) we see that these differences are in both cases biometrically essential. Hence it follows that the separate

females show here, as to the weight of their offspring, real individual differences. The inspection of the whole material of all females together recorded in Table 8 and Text-figure 1 evidences a certain distinct dominance of the Silver breed in the cross examined. But in respect to the above mentioned individual differences of the  $F_1$  offspring produced by separate females, no real importance ought to be ascribed here to the preponderance of the heavier breed.

The variability of weight of new-born hybrids twice proved larger than that of the corresponding pure Silver specimens, in the two remaining mothers being smaller (Table 11). But, as we may infer from Table F, in all cases the differences were spurious, *i.e.* the variability of weight in  $F_1$  was unchanged.

*The  $F_2$  generation.*

The biometrical data of Tables 11 and G show that the average weight of the  $F_2$  new-borns of this cross did in general not undergo any essential change as compared with the  $F_1$  generation, although we may remark here a certain shifting towards the lighter breed, analogous to that ascertained by Punnett and Bailey (24) in full-grown  $F_2$  rabbits (cf. also Tables 6, 7 and 8, as well as Text-figure 1). The variability of new-born weight is essentially larger in  $F_2$  than in the corresponding  $F_1$  generation (cf. Tables 11 and G). The only exception is represented by the offspring of ♀ 6<sub>1</sub> in which the increase has no sufficient biometrical basis. (The daughters of ♀ 5, ♀ 6, etc., were called No. 5<sub>1</sub>, 6<sub>1</sub>, etc.)

TABLE G.

*Differences of the average weights and of the coefficients of variability of weight between the  $F_2$  and  $F_1$  offspring from Silver ♀ × Himalayan ♂ cross.*

No. corresponding to each mother	Differences of the average weights		Differences of the coefficients of variability	
	Difference ± probable error	Difference: probable error	Difference ± probable error	Difference: probable error
5 <sub>1</sub>	-1.95 ± 1.72	1.1	12.76 ± 2.88	4.4
6 <sub>1</sub>	0.24 ± 1.34	0.2	6.41 ± 2.28	2.8
29 <sub>1</sub>	-6.29 ± 1.30	4.8	11.75 ± 2.10	5.6
92 <sub>1</sub>	-4.43 ± 1.54	2.9	12.94 ± 2.45	5.3

I may here remark that the Himalayan new-borns extracted in  $F_2$  of this cross were heavier than the pure-bred Himalayans, weighing in separate experiments on the average  $42.75 \pm 1.30$ ,  $43.57 \pm 1.46$ ,  $42.10 \pm 1.97$  and  $44.22 \pm 2$  grams respectively. This may easily be proved by the items of Tables 1, 7, 8, 9 and 11, as well as from Text-figure 1. Moreover an exact study of Tables 7 and 8 would show that in no case

## 254 *Inheritance of the Weight of New-born Rabbits*

are the extracted Himalayans lighter than the remaining specimens from  $F_2$ .

### CONCLUSIONS AND SUMMARY.

From the above experiments it follows that while in the mating Himalayan ♀ × Silver ♂ the offspring of all the examined females have a typical intermediate weight between the new-born weights of the two crossed breeds, in the reciprocal mating Silver ♀ × Himalayan ♂ the  $F_1$  generation from certain females is heavier than that of either parental breed. An analogous inequality may, however, also be noticed in the experiments made by several investigators on full-grown rabbits. Castle (3), who has formerly in general assumed intermediate weight of  $F_1$ , recently records the intermediate weight only in the offspring of breeds differing considerably as to weight. When using breeds which differ but slightly he ascertained a distinct phenomenon of heterosis. Punnett and Bailey (24) have in general always ascertained intermediate weight of  $F_1$ , in contrast to Davies (4), who emphasises shifting of the  $F_1$  towards the lighter breed. Similar discrepancies are noticed in respect to other mammals. King (15) lays stress on the fact that the offspring resulting from the cross between the lighter albinos and the more heavy Norwegian rats seem to be of strictly intermediate weight. The same follows from the data given by Eckles (6) for calves. Hammond (8), on the contrary, ascertained in calves shifting of the weight of  $F_1$  in the direction of the lighter breed. The same author, Hammond (10), sees that the first hybrid generation of various breeds of sheep in certain crosses approach to the weight of the heavier breed, and in certain matings they even exceed the heaviest specimens employed to the crosses<sup>1</sup>. On examining the weight of new-born, Castle (2) arrives at the conclusion that in crosses between *Cavia cutleri* and the inbred race of his guinea-pigs, the weight of the  $F_1$  hybrids somewhat surpasses both parental breeds. It is obvious that our knowledge of the body weight of  $F_1$  hybrids is far from being complete. It has been ascertained in a former paper on the offspring of Himalayan females mated by both Himalayan and Silver sires during one rutting-time, that the essential difference of weight of hybrid new-born as compared with that of Himalayans is maintained in spite of the simultaneous development of both kinds of fetuses in one mother (Kopeć (20)). In the light of these experiments it becomes most evident that the  $F_1$  weight is primarily determined by genetic factors.

<sup>1</sup> Both Mumford (23) and Humphrey and Kleinheinz (11) believe that only the breed of the ewe has an influence on the weight of the offspring, the weight of the ram being of no importance. The data of Hammond (10) do not seem to support such opinion.

From our experiments it follows that in both crosses the variability of weight was as a rule essentially greater in  $F_2$  than in  $F_1$ . We may hence infer, in concordance with the opinion of MacDowell (22) and with Lang's (21) known considerations, that the weight undergoes in  $F_2$  segregation by the interaction of polymeric factors. Such a supposition finds further support in the behaviour of the  $F_3$  offspring from the few  $F_2$  females which I succeeded in examining. The offspring of separate  $F_2$  females differed considerably as to their respective weight, *i.e.* these females underwent segregation so far as weight of their new-borns is concerned. This opinion is also corroborated by my observations that variability of weight in  $F_1$  is not larger than in  $P$  generation, and that variability in  $F_3$  is considerably decreased as compared with  $F_2$ . As to the average weight of  $F_2$ , it must be ascertained that it does not differ essentially in Himalayan ♀ × Silver ♂ cross from that of the  $F_1$  new-borns, giving all appearances of Castle's "blending" inheritance; the weight of  $F_2$  new-born of the reciprocal cross evidences rather a certain shifting in the direction of the lighter breed, analogous to the results obtained by Punnett and Bailey (24).

On examining our two crosses we have found that the average weight of the Himalayan new-born extracted in  $F_2$  does not differ essentially from the weight in the remaining young of the same generation. On the other hand, the Himalayan extracted in the  $F_2$  generation are heavier than the pure-bred Himalayans. The pattern and the weight of new-borns therefore undergo segregation independently of each other. By means of mating Himalayan females with the heavier Silver breed we may in time obtain Himalayan does giving birth to heavier new-born than those produced by the pure-bred mothers.

From the results of the foregoing inquiry we arrive at the following conclusions:

1. The Himalayan ♀ × Silver ♂ cross gives  $F_1$  new-born, the average weight of which is intermediate between the new-born weights of both breeds. Variability of weight of such  $F_1$  hybrids is maintained the same as in pure Himalayan young.

The average weights of the  $F_2$  new-born of such cross do not exhibit any significant changes as compared with  $F_1$ . The variability of weight evidenced by  $F_2$  is much larger than that in  $F_1$ .

Apart from decreased variability of weight in the  $F_3$  generation, as compared with  $F_2$ , a distinct segregation has been ascertained. The majority of the  $F_2$  females produce  $F_3$  new-born having almost the same weight as the new-born of the  $F_2$  generation; in one case the  $F_3$



## 256 *Inheritance of the Weight of New-born Rabbits*

specimens weighed essentially more, in another essentially less than the  $F_2$  new-born.

2. In the reciprocal cross Silver ♀ × Himalayan ♂ no distinct regularity can be ascertained in the weight of the  $F_1$  new-born. Here again the variability of weight of  $F_1$  new-born does not undergo any changes dependently on analogical variability of the Silver breed.

The weight of  $F_2$  new-born shows a certain decrease as compared with  $F_1$ . The variability of weight was in  $F_2$  essentially greater than in the first hybrid generation.

3. In agreement with MacDowell's opinion, based on his experiments with full-grown rabbits, the inheritance of body weight in the new-born stage may also be considered as Mendelian, governed by polymeric factors.

4. The Himalayan new-born extracted in  $F_2$  from both crosses are heavier than the pure-bred Himalayan specimens. In both cases the weight of the extracted Himalayans was the same as that of the remaining non-Himalayan components of the  $F_2$  generation. The separate breed characters, such as pattern and weight, consequently undergo segregation independently from each other.

The investigations of which an account is given above have been in part carried out by means provided by the Department of Science of the Ministry of Instruction.

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258 *Inheritance of the Weight of New-born Rabbits*

TABLE 1.

Throughout Tables 1-7 the items denote the weights of separate new-born in gms.

*Himalayan* ♀ × *Himalayan* ♂ (No. 7).

ex ♀ 8	ex ♀ 10	ex ♀ 11	ex ♀ 25	ex ♀ 36	ex ♀ 77	ex ♀ 118
21	28	29	28	30	29	27
27	29	29	29	32	30	28
28	31	29	31	33	32	30
29	31	31	31	33	34	31
30	32	31	32	33	35	33
30	33	32	34	33	35	34
30	33	33	34	34	37	35
30	35	34	35	34	37	35
30	35	34	36	37	38	36
30	37	35	36	39	38	36
31	37		37	41	39	37
31	37		37	46	40	37
31	38		38	47	42	38
32	38		38	47	43	40
33	39		39	48	43	42
34	40		39		43	
34	40		40		44	
36	41		40		45	
38	41		40		45	
39	41		41		46	
39	41		42		46	
40	42		42		46	
	42		42		47	
	42		43		47	
	43				48	
	44				49	
	45					

TABLE 2.

*Silver* ♀ × *Silver* ♂.

ex ♀ 5	ex ♀ 6	ex ♀ 29	ex ♀ 92
40	41	26	36
41	42	32	37
42	42	36	37
42	43	36	39
42	43	37	39
42	43	37	39
43	43	38	39
45	44	38	40
46	44	39	41
46	44	39	41
46	45	39	42
47	45	39	43
47	46	39	43
48	46	40	46
48	47	41	47
48	48	41	48
49	48	42	51
49	49	42	51
49	49	43	53
49	49	44	53
50	49	44	
51	50	45	
51	50	46	
53	50	47	
53	51	48	
55	54	48	
58	55	50	

TABLE 3.

*F*<sub>1</sub> generation from *Himalayan* ♀ × *Silver* ♂ (No. 44).

ex ♀ 8	ex ♀ 10	ex ♀ 11	ex ♀ 25	ex ♀ 36	ex ♀ 77	ex ♀ 118
34	38	32	38	36	32	47
34	38	33	38	36	35	48
37	40	35	38	37	35	48
38	40	36	40	37	36	49
38	40	36	41	38	36	50
39	42	37	42	39	37	51
39	42	39	43	39	41	55
40	44	40	45	39	42	56
40	45	42	47	39	43	57
40	45		48	40	45	
41	45		48	40	46	
41	46		49	41	46	
42	47			42	46	
42	47			42	47	
42				43	50	
43				44	52	
44				45	53	
46				46	56	
46						
47						
47						
48						

TABLE 4.

*F*<sub>2</sub> generation from *Himalayan* ♀ × *Silver* ♂.

Items in italics refer to the Himalayan new-born extracted in *F*<sub>2</sub>. ♀ 8<sub>1</sub> derives from the Himalayan female No. 8, ♀ 10<sub>1</sub> from the Himalayan female No. 10, etc.

ex ♀ 8 <sub>1</sub>	ex ♀ 10 <sub>1</sub>	ex ♀ 11 <sub>1</sub>	ex ♀ 25 <sub>1</sub>	ex ♀ 36 <sub>1</sub>	ex ♀ 77 <sub>1</sub>	ex ♀ 118 <sub>1</sub>
23	27	20	18	22	25	30
26	32	26	35	28	32	32
31	36	31	41	29	33	32
32	37	35	41	29	34	33
33	39	36	42	30	34	33
33	40	37	42	31	35	34
33	40	37	43	32	35	34
33	40	38	44	33	35	37
33	41	38	45	35	35	38
34	41	39	46	35	36	38
36	41	40	46	36	36	39
40	43	40	47	37	36	41
41	43	40	47	37	38	41
41	43	42	49	38	39	42
41	44	42	49	43	39	42
42	44	42	49	47	39	42
42	47	43	50	55	39	42
42	47	44	50		40	43
42	48	44	51		40	43
43	49	45	52		40	44
43	49	45	55		41	44
44	52	45	56		41	44
46	52	46	56		41	44
46	54	47	58		41	45
46	58	48			42	45
47		49			42	46
48		50			43	47
48		54			43	48
49		57			44	49
50		60			44	51
50					45	60
52					46	
52					47	
52					50	
52					52	
58					54	

TABLE 5.

*F<sub>3</sub> generation from Himalayan ♀ × Silver ♂.*Females No. 109–No. 138 were produced by ♀ 36<sub>1</sub> × ♂ 36<sub>1</sub>.

ex ♀ 109	ex ♀ 112	ex ♀ 121	ex ♀ 125	ex ♀ 126	ex ♀ 130	ex ♀ 138
28	35	32	28	25	28	23
30	36	34	28	26	28	25
31	38	34	30	28	33	25
33	38	34	30	28	35	26
37	39	34	31	28	35	27
38	40	35	34	35	37	28
38	40	35	34	36	38	28
40	41	37	35	37	38	29
41	42	37	37	38	39	30
42	43	37	38	39	41	30
46	45	38	38	39	45	30
47	45	38	39	39		30
48	45	40	44	40		31
48	45	40	46	40		31
52	46	41	47	42		32
	47	42	47	42		32
	48	43		43		33
	49	43		43		34
		45		47		36
	53	45		47		36
		46				36
						38

TABLE 6.

*F<sub>1</sub> generation from Silver ♀ × Himalayan ♂.*

ex ♀ 5	ex ♀ 6	ex ♀ 29	ex ♀ 92
33	34	39	39
38	36	44	39
40	36	45	41
42	37	45	42
43	37	46	43
45	38	46	43
45	39	46	43
45	39	47	44
45	39	49	44
45	42	50	45
46	42	52	46
47	42	53	47
49	43	57	48
50	43		49
	44		52
	44		
	45		
	46		
	47		
	49		
	50		



TABLE 9.

*Biometrical constants referring to the offspring of each female separately in the Himalayan ♀ × Silver ♂ cross.*

♀ 8<sub>1</sub> derives from the Himalayan female No. 8, 10<sub>1</sub> from the Himalayan female No. 10, etc.

Material	No. corresponding to each mother	Average weight and its probable error $A \pm E_A$	Coefficient of variability of weight and its probable error $C \pm E_C$	$\pm \sigma$	Average number of young in the litter	Total number of specimens
Himalayan ♀ × Himalayan ♂	8	31.95 ± 0.64	14.02 ± 1.43	4.48	5.5	22
	10	37.59 ± 0.62	12.61 ± 1.16	4.74	4.5	27
	11	31.70 ± 0.48	7.13 ± 1.08	2.26	5.0	10
	25	36.83 ± 0.59	11.73 ± 1.14	4.32	4.8	24
	36	37.80 ± 1.11	16.85 ± 2.07	6.37	5.0	15
	77	40.69 ± 0.76	14.18 ± 1.33	5.77	4.3	26
	118	34.60 ± 0.73	12.20 ± 1.50	4.22	5.0	15
Himalayan ♀ × Silver ♂	8	41.27 ± 0.57	9.57 ± 0.97	3.95	4.4	22
	10	42.79 ± 0.58	7.46 ± 0.96	3.19	4.6	14
	11	36.67 ± 0.73	8.84 ± 1.41	3.24	4.5	9
	25	43.08 ± 0.82	9.77 ± 1.35	4.21	4.0	12
	36	40.17 ± 0.48	7.47 ± 0.84	3.00	6.0	18
	77	43.22 ± 1.11	16.20 ± 1.82	7.00	4.5	18
	118	51.22 ± 0.85	7.42 ± 1.18	3.80	4.5	9
	8 <sub>1</sub>	41.49 ± 0.92	19.50 ± 1.57	8.09	5.0	35
	10 <sub>1</sub>	43.48 ± 0.93	15.92 ± 1.52	6.92	5.0	25
	11 <sub>1</sub>	42.00 ± 1.01	19.60 ± 1.71	8.23	6.0	30
	25 <sub>1</sub>	46.33 ± 1.13	17.68 ± 1.72	8.19	4.8	24
	36 <sub>1</sub>	35.12 ± 1.27	22.18 ± 2.57	7.79	5.7	17
	77 <sub>1</sub>	39.78 ± 0.67	15.06 ± 1.20	5.99	5.1	36
	118 <sub>1</sub>	41.39 ± 0.78	15.63 ± 1.34	6.47	5.2	31

TABLE 10.

*Biometrical constants referring to the F<sub>3</sub> generation from Himalayan ♀ × Silver ♂ (all females were produced by ♀ 36<sub>1</sub> × ♂ 36<sub>1</sub>).*

No. corresponding to each mother	Pattern of the female	Numerical relation between the greyish-blue and the pink new-borns	Average weight and its probable error $A \pm E_A$	Coefficient of variability of weight with its probable error $C \pm E_C$	$\pm \sigma$	Average number of young in the litter	Total number of specimens
109	Black-silver	15 : 0	39.93 ± 1.28	18.36 ± 2.26	7.33	5.0	15
112	Himalayan	0 : 20	43.35 ± 0.76	11.70 ± 1.25	5.07	5.0	20
121	Black-silver	20 : 1	38.57 ± 0.66	11.59 ± 1.21	4.47	5.3	21
125	"	12 : 4	36.63 ± 1.11	17.99 ± 2.15	6.59	5.3	16
126	"	11 : 9	37.10 ± 1.02	18.19 ± 1.94	6.75	5.0	20
130	Himalayan	0 : 11	36.09 ± 1.04	14.22 ± 2.05	5.13	5.5	11
138	Black-silver	0 : 22	30.45 ± 0.58	13.14 ± 1.34	4.00	4.5	22

TABLE 11.

*Biometrical constants referring to the offspring of each female separately in the Silver ♀ × Himalayan ♂ cross.*

♀ 5<sub>1</sub> derives from the Silver female No. 5, ♀ 6<sub>1</sub> from the Silver female No. 6, etc.

Material	No. corresponding to each mother	Average weight with its probable error $A \pm E_A$	Coefficient of variability of weight with its probable error $C \pm E_C$	$\pm \sigma$	Average number of young in the litter	Total number of specimens	
Silver ♀ × Silver ♂	5	47.41 ± 0.58	9.43 ± 0.87	4.47	4.5	27	
	6	46.67 ± 0.48	7.95 ± 0.73	3.71	4.5	27	
	29	40.59 ± 0.67	12.81 ± 1.18	5.20	5.4	27	
Silver ♂	92	43.25 ± 0.83	12.79 ± 1.36	5.53	5.0	20	
Silver ♀ × Himalayan ♂	$F_1$	5	43.78 ± 0.80	10.14 ± 1.29	4.44	4.6	14
		6	41.52 ± 0.66	10.74 ± 1.12	4.46	5.2	21
		29	47.61 ± 0.86	9.64 ± 1.28	4.59	4.3	13
		92	47.50 ± 0.85	10.28 ± 1.27	4.88	5.0	15
	$F_2$	5 <sub>1</sub>	41.83 ± 1.52	22.90 ± 2.57	9.58	4.5	18
		6 <sub>1</sub>	41.76 ± 1.17	17.15 ± 1.98	7.16	4.3	17
		29 <sub>1</sub>	41.32 ± 0.97	21.39 ± 1.66	8.84	5.4	38
		92 <sub>1</sub>	43.07 ± 1.28	23.22 ± 2.09	10.00	4.7	28





# EXPERIMENTS WITH CERTAIN PLUMAGE COLOUR AND PATTERN FACTORS IN POULTRY.

By W. E. AGAR, F.R.S.

*Professor of Zoology, University of Melbourne.*

THESE experiments were primarily undertaken with the view of testing linkage and crossing-over in the two sex-linked genes for bar and silver with allelomorphs non-bar and gold. When the seexperiments were begun I was unaware that similar experiments had been undertaken by Goodale (1917) and Haldane (1921). During the course of the experiment a third paper dealing with the same genes was published by Serebrovsky (1922). Haldane appears to be the only one who has determined the cross-over value, however, and as, for the establishment of these values, large numbers are essential, it seemed well worth while to complete the experiment in spite of its having been done before. Moreover, although the above-mentioned workers used the same two sex-linked genes as myself, the breeds of fowls used were in part different, and some information regarding certain other characters has been incidentally obtained and may be worth recording. Unfortunately, the extent of the experiment was limited by restricted space.

## THE MAIN CHARACTERS CONCERNED.

(1) Barring of the feathers (*B*, *b*). This is the well known sex-linked gene, present in its most familiar form in the barred Plymouth Rocks, where it causes the restriction of the black pigment to parallel bars across the feathers. It is generally stated that this factor acts on black pigment alone, but this is certainly not the case. I find that it has the same effect on gold (or red), restricting this colour in the same way as it restricts black in the barred Plymouth Rocks, so that the feathers show alternating gold and white bars.

(2) Silver or white (*S*) dominant over gold or red (*s*).

(3) A gene upon which depends the distribution of black pigment.

This is the gene identified by Dunn (1922) and named by him *E<sup>m</sup>*. In its dominant form it causes the extension of the black pigment to all

parts of the plumage (Plymouth Rocks, Black Orpingtons). When present in its recessive form, the pigment is restricted to hackle, wing and tail quills, giving the well-known Columbian pattern. The amount of black pigment developed by  $e^m$  birds is however plainly influenced by other factors also. The Columbian of the fanciers, *e.g.* Columbian Wyandottes, represents a more or less middle position in this respect. In the Rhode Island Reds, which are also  $e^m$ , the black is much more restricted, appearing as a few minute black specks in the hackle, with larger patches on the wing and tail quills. The other end of the scale is represented by certain homozygous  $e^m$  birds obtained in the  $F_3$  of my experiments, in which the black was so spread out from hackle and tail that the white (or red) portion of the birds was almost confined to a broad irregular belt round the middle of the body. Extended black is nearly epistatic over silver and gold.

(4) A fourth character which enters conspicuously into one of the experiments is *lacing*, *i.e.* the presence of a black edge to an otherwise silver or gold feather. Although this gene has entered into many experimental crosses, it does not seem to have been specially studied. As will be shown below, its behaviour in my experiments can be satisfactorily accounted for on the supposition that it depends upon a single factor, which in its recessive form ( $l$ ) produces lacing, and in its dominant form ( $L$ ), absence of lacing. Lacing is hypostatic to extended black, and nearly epistatic over barring.

(5) Besides the above, many subsidiary genes are of course involved in these crosses, which produce modifications of the typical conditions.

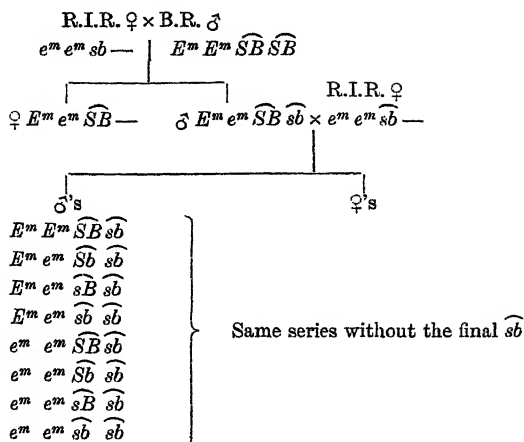
The breeds used:

- |                                  |   |
|----------------------------------|---|
| 1. Barred Plymouth Rock (B.R.)   | $\sigma = E^m E^m ll \quad \widehat{SB} \widehat{SB}$ |
|                                  | $\text{♀} = E^m E^m ll \quad \widehat{SB} \text{—}$   |
| 2. Rhode Island Red (R.I.R.)     | $\sigma = e^m e^m LL \widehat{sb} \widehat{sb}$       |
|                                  | $\text{♀} = e^m e^m LL \widehat{sb} \text{—}$         |
| 3. Golden Laced Wyandotte (G.W.) | $\sigma = e^m e^m ll \quad \widehat{sb} \widehat{sb}$ |
|                                  | $\text{♀} = e^m e^m ll \quad \widehat{sb} \text{—}$   |

#### EXPERIMENT 1. *The cross R.I.R. ♀ × B.R. ♂.*

Since the way in which this cross was carried out insured that all individuals should carry at least one  $L$  gene, no laced birds could appear. This gene is therefore left out of the formulae.

Scheme of the experiment:



#### DESCRIPTION OF THE PHENOTYPES.

$F_1$  ♂. General aspect that of a barred Plymouth Rock, but with a good deal more white on shoulder and breast, where the black bars are reduced, irregular or wanting. The recessive gold or red shows as isolated gold feathers, especially in hackle and saddle. In addition there are a few well marked chestnut (see below) flecks, especially on flights and wing coverts.

$F_1$  ♀. Very like the B.R. The dark bars are somewhat broader than in the typical B.R., giving a darker aspect to the bird as a whole. No trace of gold.

$F_2$ — $E^m e^m \widehat{SB} \widehat{sb}$ . The description applied to the  $F_1$  male applies equally to this. The corresponding female departs only in minor points from the typical B.R.

$E^m e^m \widehat{SB} \widehat{sb}$ . These can best be described as unbarred black fowls with white markings. The recessive gold shows as gold markings on hackle and saddle. The corresponding female is similar, without the gold.

$E^m e^m \widehat{sB} \widehat{sb}$ . Like the B.R., but with much gold on the hackle and throat, and a gold flush over the body generally. This is due to a slight gold tinge on the outer edges of the feathers. (This description applies to the female, no male of this class having been obtained.)

$E^m e^m \widehat{sb} \widehat{sb}$ . Unbarred blacks with gold markings conspicuous both in male and female.

$e^m e^m \widehat{SB} \widehat{sb}$ . Columbian pattern, with black much more restricted than in typical Columbian. Black feathers are barred.

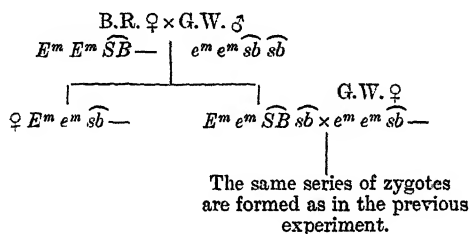
$e^m e^m \widehat{Sb} \widehat{sb}$ . Like the preceding class, only unbarred.

$e^m e^m \widehat{sB} \widehat{sb}$ . Best described as a barred R.I.R. In the parts where the Columbian black is developed the white bars alternate with black, and over the rest of the body with red.

$e^m e^m \widehat{sb} \widehat{sb}$ . Practically identical with R.I.R., except that many of them have more black on the Columbian pattern than the typical R.I.R.

#### EXPERIMENT 2. *The cross B.R. ♀ × G.W. ♂.*

All the individuals of this pedigree were homozygous  $ll$ , and were therefore potentially laced. As, however, lacing is hypostatic to extended black (introduced by the B.R.), only those birds homozygous for  $e^m$  are laced. Since  $ll$  is the same in all birds,  $l$  is left out of the formulae.



#### DESCRIPTION OF PHENOTYPES.

These are sufficiently like the phenotypes of the last experiment to enable us to dispense with special description, except

(1) The  $F_1$  female's are of course quite different from the corresponding forms in the previous experiment, since in the present cross the barring factor was introduced by the female parent, and therefore does not appear in the  $F_1$  hens. These are solid black, with the hypostatic gold showing through here and there as minute gold streaks.

(2) The  $e^m e^m$  birds, which in the previous experiment showed the Columbian pattern on a silver or gold background, are here silver or gold laced, since the absence of the epistatic extended black allows the lacing to appear. There are four classes of  $e^m e^m$  birds, viz.:

- (1) Silver, barred, laced.
- (2) Silver, unbarred, laced.
- (3) Gold, barred, laced.
- (4) Gold, unbarred, laced.

Of these Nos. 2 and 4 are indistinguishable from the typical silver and gold laced Wyandottes. Nos. 1 and 3 are laced over the greater part of the body, but the black edgings of the feathers are much thinner than in the unbarred, laced birds. The barring only appears in the hackle, flanks, saddle, tail, and under wing coverts, *i.e.* those parts in which the lacing is absent or least developed in the pure laced Wyandottes. Lacing is therefore nearly completely epistatic over barring.

#### LINKAGE AND CROSSING-OVER.

Both in the R.I.R.  $\times$  B.R. and the B.R.  $\times$  G.W. experiments,  $F_1$  male's of the composition  $\widehat{SB} \widehat{sb}$  were crossed back with female's of the composition  $\widehat{sb}$ . Two males were used in each case.

There was no difficulty in distinguishing between the four types of offspring in the Wyandotte experiment. Unfortunately, only 25 chickens were reared in this experiment, which gave the proportions:

$$SB \ 10, \quad Sb \ 5, \quad sB \ 5, \quad sb \ 8.$$

giving a cross-over value of 35.7 per cent.

In the R.I.R. cross some dubiety attached to the silver birds of the  $e^m e^m$  (Columbian) class. Naturally in these birds barring can only appear on those parts of the feathers where black pigment is present, and in many of them the black was so restricted that barring had small opportunity to display itself, and certain of the birds had to be recorded as doubtful in respect of barring.

There were 15 birds in this class, and they were arranged in order from those certainly barred to those certainly unbarred. One about the middle of the series (recorded as "probably barred") was tested by breeding, but proved to be unbarred. The only safe thing to do, therefore, is to leave this class out of account altogether, in spite of the consequent reduction of the total number available.

The remaining birds are classified as follows:

	<i>SB</i>	<i>Sb</i>	<i>sB</i>	<i>sb</i>
$E^m$ birds	7	9	10	4
$e^m$ "	?	?	7	19
	7	9	17	23

Assuming that *SB* and *sb* in the  $e^m$  class would have followed the same linkage value as in the other classes, we have 26 cross-overs out of a total of 56, or a cross-over value of 46.4 per cent.

Goodale (preliminary report, 1917) and Serebrovsky (1912) both state that crossing-over takes place between these two genes, but give no

figures. Haldane (1921) with a total of 78 birds, finds a cross-over value of 34.6 per cent., practically equal to my value from the G.W. experiment. The irregularity of the numbers in my R.I.R. experiment detracts from the reliability of the high value 46.4.

(*Note.* Haldane found an excess of silver over gold in his experiments of 47 : 31. My experiments gave 46 silver : 53 gold—equality being expected.)

Further experiments on the B.R. and G.W. cross, where all the phenotypes are easily distinguished, are in progress.

#### NOTES ON SOME MISCELLANEOUS POINTS.

##### 1. *The Barring Factor.*

Punnett and Pease (1921) have recently drawn attention to the existence of two different barring factors in poultry—one, the well-known sex-linked gene, best exemplified by the barred Plymouth Rock, and the other an autosomal gene found in Hamburgs and Campines (here generally known as pencilling). The two common forms of pencilled Hamburgs are the gold and silver, in which bars of one of these colours alternate with black. A third form, the Chamois, has alternate bars of gold and white. The authors show that the Chamois is to be derived from the gold pencilled form by a dominant factor which inhibits the development of black, so that the black bars of the gold or silver pencilled Hamburg correspond to the white bars of the Chamois. It is interesting to note that the combination of the sex-linked barring factor with the recessive restriction factor  $e^m$  produces a phenotype very similar to the Chamois—namely, alternating gold and white bars—only here the white bars do not correspond with the black bars of the B.R., but with the white.

The factor  $B$ , it is to be noted, inhibits the production not only of melanic pigment (Haldane, Hertwig) but also of gold.

It is also noteworthy that although, as my experiments show (and as Punnett and Pease have observed), the B.R. carries silver, the silver bars on the plumage are not an expression of this fact, for similar silver coloured bars appear on the plumage of the barred golds, which, of course, do not carry silver.

##### 2. *Lacing.*

The evidence for the interpretation of lacing given above is contained in the following experiments. In these we need only consider

the two factors  $E^m$  and  $L$ . The composition of the three breeds is then:

B.R.  $E^m E^m ll$ —unlaced because  $l$  is hypostatic to  $E^m$ .

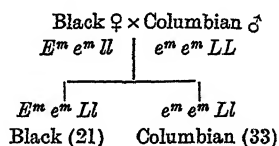
R.I.R.  $e^m e^m LL$ —unlaced owing to presence of  $L$ .

G.W.  $e^m e^m ll$ —laced.

(1) In experiment 1 (R.I.R.  $\times$  B.R.)  $\times$  R.I.R. no laced individuals appeared, because all were  $LL$  or  $Ll$ .

(2) In experiment 2 (B.R.  $\times$  G.W.)  $\times$  G.W. all the birds contain  $ll$ , and are therefore potentially laced, but as half of them contain epistatic  $E^m$ , only the other half (the  $e^m e^m$  birds) show the lacing. (Actual figures: laced 13, unlaced 12.)

(3) The  $F_1$  black hens from the B.R.  $\times$  G.W. cross (composition  $E^m e^m ll$ ) were crossed with an  $F_2$  Columbian male from the (R.I.R.  $\times$  B.R.)  $\times$  R.I.R. cross. The composition of this male might have been either  $e^m e^m Ll$  or  $e^m e^m LL$ , the result of the experiment showing that it must have been the latter. This cross becomes therefore:



Equality of the two types was of course expected on the theory. Among the Columbians one bird showed a fair amount of lacing, presumably due to incomplete dominance of  $L$ .

### 3. Spangling.

In the cross (R.I.R.  $\times$  B.R.)  $\times$  R.I.R. four males out of 34 showed well-developed spangles on the breast. None of the females showed it. Lefevre (1916) finds that spangling (as in the spangled Hamburgs) is a sex-linked factor.

### 4. Chestnut.

In the experiment (R.I.R.  $\times$  B.R.)  $\times$  R.I.R. there appeared in the males of  $F_1$ , and in several males and a few females of  $F_2$ , a colour which I cannot identify with any of those which have been genetically investigated so far, and which might provisionally be called chestnut. In hue it approaches very near to the R.I.R. colour, but is rather browner. It appears in the form of irregular spots or flecks, chiefly across the back and wing coverts, and appears on some females carrying  $S$ , showing that it is



genetically as well as phenotypically distinct from *s*. It varies very much in amount, from a few very small flecks to a saddle-shaped patch across the back. Its presence is difficult to determine, both in *E<sup>m</sup>* and in *ss* birds, but it is a very conspicuous feature on White Columbians.

Circumstances did not allow of detailed investigation of this character.

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## RESEARCHES ON ANIMAL CHIMAERAS

BY V. ISSAYEV.

*Laboratory for Genetics and Experimental Zoology,  
Petrograd University (Russia).*

(With Nine Plates and Six Text-figures.)

## CONTENTS.

## I.

	PAGE
1. Introduction . . . . .	274
2. Material and methods . . . . .	276
3. Natural sectorial chimaeras . . . . .	280
4. Artificial sectorial chimaeras . . . . .	281
5. Chimaeras as results of insertion . . . . .	288
6. Another instance of an insertion chimaera . . . . .	297
7. Another example of the regulation of a chimaera . . . . .	305

## II.

8. The budding of cytomictical chimaeras . . . . .	308
9. The first generation, $G_1$ . . . . .	311
10. The second generation, $G_2$ . . . . .	316
11. The third generation, $G_3$ . . . . .	321
12. The fourth generation, $G_4$ . . . . .	329
13. The fifth to seventh generations, $G_5-G_7$ . . . . .	331
14. The segregation of other cytomictical chimaeras . . . . .	334
15. Sexual generation of oligactoids (?) . . . . .	338

## III.

## General conclusions:

1. Organic regulations . . . . .	338
2. Chimerality . . . . .	339
3. Oligactoids . . . . .	341
4. Vegetative segregation of the oligactoids . . . . .	343
5. The origin of oligactoids . . . . .	344
6. The problem of the shape of oligactoids . . . . .	346
7. The importance of $I$ -cells in the regulation process . . . . .	346
8. The soma and the germ-plasm . . . . .	348
9. About death and immortality . . . . .	349
10. About somatic mutations . . . . .	349

THE existing abnormal difficulties in printing scientific works in Russia have delayed the publication of the results of my last three years' researches.

A short summary of my recent investigations appeared in 1923 in the *Biol. Zentralblatt*, Vol. XLIII.

## I.

## 1. Introduction.

"Chimaera" is the name given to organisms of a specially complex nature, formed of the tissues of two different organisms—tissues of different genetic origin.

Chimaeras can be whole organisms, but, as a rule, we have to deal with separate organs of chimeric structure. They are met with in nature, and then they owe their existence to somatic mutation in certain plant cells that have undergone that process. But chimaeras are also obtained during experimental processes, when grafting one plant upon another.

The outward appearance of chimeric organisms varies; they either retain the look of their parental component parts, or more frequently, they present something akin in form to both parental components. Whatever may be the relative position of the tissues (whether one beside, or one above the other), whatever their respective appearance—only one fact is of importance: notwithstanding the close adhesion of the tissues and their forming one physiological whole, with a common nutritive fluid, there is neither a fusion of the cells of the different tissues, nor even the slightest sign of modifying influence of one component on the structure of the other. The tissues retain entirely their typical genetic purity. The intermediate form of the chimeric organism is absolutely non-transmissible to the next generation; and whenever one of its components can be extracted it will continue to multiply only in a pure form. The internal relations of chimeric objects have been very aptly designated by the term "graft-symbiosis" (Buder, 1911).

If, however, we wish to change a graft-chimaera into a true vegetative graft-hybrid, it is necessary that its intermediate character should become hereditary.

The problem of producing such hybrids was already stated by Darwin, and can be now looked upon as fairly solved. After *Cytisus Adami* the first artificial plant chimaeras were, it seems, obtained in England. The earliest literary indications of their production found by me point to the years 1865 to 1869. Into the science of genetics the conception of chimaeras and the very term were introduced in 1907 by Winkler, who fancied he saw in the organisms which he had obtained true graft-hybrids. Winkler's mistake was cleared up by Baur in 1909. Of late years a great number of authors have studied and described a whole series of chimaeras and chimeric organs in the most varied plants. Thus, for instance, Buder (1911), Winkler (1912, 1914, 1916), J. Meyer (1915), Burgeff (1915),

Bateson (1916, 1919, 1921), Lotsy (1918), Babcock (1918), Correns (1919), Åkerman (1920), Noack (1922).

Real animal chimaeras have not yet been discovered in nature.

When in 1921 I obtained the first sectorial and mosaic chimaeras through grafting two species of Hydras—*Hydra vulgaris* and *H. oligactis*—I thought that these were indeed the first animal chimaeras ever obtained in experiments. The study of the literature on the subject soon showed me my mistake. Organs of chimeric structure were first obtained in T. H. Morgan's Laboratory by Harrison in 1898. One of his diagrams on the subject illustrates the tadpole *Rana palustris*; in the tail under the light coloured epidermis there spreads an inner layer of the dark cells of *Rana sylvatica*. The year after, in 1899, T. Morgan examined and verified Harrison's conclusions; no influence whatever of the tissue of one species upon the other was found.

In 1900 Miss Peebles obtained buds during the regeneration of serially grafted green Hydras that showed some likeness to sectorial chimaeras. Yet it is difficult to speak of true chimaeras in this case, as the organisms belonged to the same species, although they were of different colour.

The same remark applies to the investigations of Garbowski (1904) who grafted portions of the eggs of the sea-urchin (*Psammechinus miliaris*) variously stained with vital colours, into a mosaic whole and investigated its further development.

True sectorial chimaeras were obtained by Bierens-de-Haan (1913). On the white pluteus of the sea-urchin *Parachinus microtuberculatus* he obtained a red sector of the tissues of the *Paracentrotus lividus*.

It was Spemann, who in 1921 first used the term "animal chimaeras" intentionally and consciously. He had succeeded in 1919 in transferring parts of tissues from the surface of a coloured germinating egg of *Triton taeniatus* to the white eggs of *Triton cristatus*, and *vice versa*. In this way were produced various organs of a special chimeric structure, such as eyes, gills, and so forth.

In 1922 there appeared Schaxel's paper. By means of transplanting the rudiments of regenerating limbs of two varieties of Axolotls—a black and a white one—he obtained chimerical limbs of a sectorial and mosaic type. But on grafting the whole small white axolotl-larva upon the large black one, he enclosed it in a black epidermis; in that way he obtained a paradoxical periclinal animal chimaera.

In the autumn of 1921 W. Goetsch found, evidently at the same time with me, a chimerical bud (of sectorial type) as the result of joining serially individuals of the colourless and the green variety of *H. viridis*.

*sim*a Pallas—a result which is analogous to that obtained in her experiments by Miss Peebles. In 1922 the same result was obtained by uniting the green variety of *H. attenuata* var. *viridescens* W. Goetsch with the brown *H. vulgaris* Pallas.

I got my sectorial, periclinal, mosaic and curious “cytomictical” chimaeras by grafting *H. vulgaris* Pallas and *H. oligactis* Pallas in the summer of 1921. The summer of 1922 was devoted by me to the special study of the genetics of these chimaeras. Previous to my experiments nothing was known of the way in which animal chimaeras multiply.

## 2. Material and Methods.

We have at our disposal but one method of obtaining chimaeras artificially—that is grafting. In order to obtain true animal chimaeras it is absolutely necessary to select individuals belonging to two different species, and therefore this operation must be referred to the category of heteroplastic transplantations.

Yet all experiments in zoology lead to the conclusion that heteroplastic fusions are seldom successful, and especially, as far as Hydras are concerned, the investigations of Wetzel (1895 and 1898) and Koelitz (1911) had proved that the union of *H. vulgaris* and *H. oligactis* succeeds for a very short time; sooner or later both components invariably parted.

In spite of this unfavourable experience I undertook a series of experiments in joining such components by grafting, and thanks to a new method which I applied, my efforts were crowned with success.

In my heteroplastic experiments I made use of the following objects:

1. *H. oligactis* Pallas, stalk-hydra, or as it is now called by P. Schulze (1917), who has classified it as a special genus, *Pelmatohydra oligactis* Pallas.

2. *H. vulgaris* Pallas, the common, stalkless Hydra. An unusually fortunate circumstance accompanied my experiments. I found a variety of this species of the Hydra (a local race) in Lake Parsolovskoye near Old Peterhof (in the vicinity of Petrograd). It was of a bright red colour—the result of the presence of carotinoid and lycopinoid matter (analyzed by Prof. Liubimenko). The tints varied from a brick red to a purple red. Owing to this circumstance the edges of the united tissues were always clearly distinguishable.

In all my attempts of the year 1922 at uniting by grafting different Hydras, I always chose the component part from the *oligactis* of pure descent which I had bred myself from one specimen taken in spring. The *H. vulgaris* I collected invariably during two years of experiments

from the same place (about two square metres in extent) on the banks of a growing peat-bog.

In the following pages I shall term this species of Hydra the *red* one, and the stalk-hydra the *brown* one.

My heteroplastic operations upon Hydras were preceded by homoplastic ones upon *P. oligactis*. These latter were undertaken in order to solve the problem of the organic individuality of Hydras. I expected to find approaches to the solution of this problem in grafting experiments. I therefore joined together *whole* animals, whole individuals. In order to distinguish this kind of experiment from the usual "transplantation" I suggested the term "complantation." At that time I applied the term "individuals" to such whole animals or portions of them as are capable of leading a separate existence and of building up a whole organism anew.

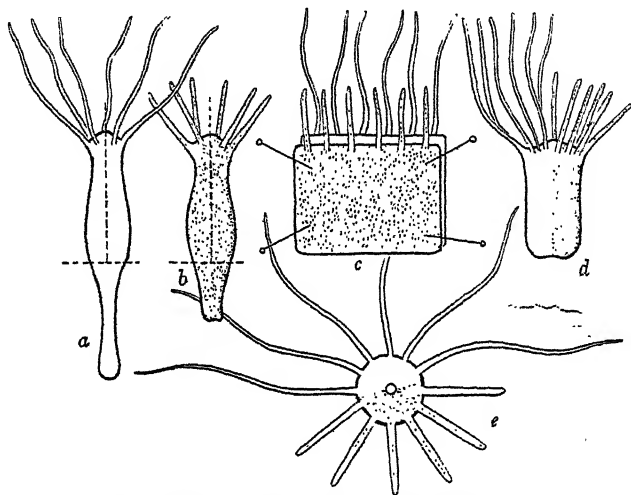
The aim of my work, however, did not lie in the preservation of the individuality of one of the components, but in its destruction. I therefore cast aside the usual method of procedure in uniting Hydras which takes its origin from Trembley's experiments in 1744 and consists in stringing together two or more Hydras in a long file on a bristle, and followed an entirely new line.

1. In specimens destined for complantation (I am speaking only of brown Hydras) I began by removing the stalk (the inactive part of the body of the Hydra) and the budding zone; the remainder presented a small cylinder open at the bottom and bearing tentacles at the top. In this cylinder I made a lengthwise incision through the body wall beginning at the mouth; I then laid it out flat so that the entodermal surface was turned towards the object-lens of the binocular. In precisely the same way I prepared the second Hydra. I then joined the entodermal surfaces of both animals and pinned their edges together with thin entomological pins. All these processes were accomplished with the help of pins and a scalpel with a long narrow end.

In about half an hour to an hour's time I was able to remove the pins; in successful cases the free side edges of the two Hydras used to stick together, and in a few hours the Hydra became round and the bottom closed up. The common mouth aperture and the hypostome were surrounded by an even crown of tentacles, but instead of the usual number of six tentacles, the united Hydra possessed twelve. This double Hydra behaved exactly like the single ones in its processes of alimentation, regeneration, budding, multiplying—but of this I intend writing in another place.

In the place of the vanished individualities there appeared one new individual. The regulating process took the visible form of a junction of tentacles pairwise, and their numerical reduction to the normal number six.

I followed exactly the same way when uniting the brown and the red Hydras, and obtained as a result the artificial sectorial chimaeras after the method of *complantation* (Text-fig. 1).



Text-fig. 1. Heteroplastic grafting-experiment (Complantation). *a* = *P. oligactis*; *b* = *H. vulgaris*; *c* = two unrolled Hydras pinned together; *d* = artificial sectorial chimaera from the side; *e* = id. from above.

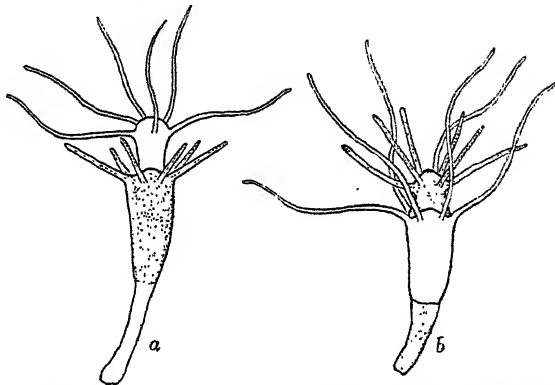
2. In another series of experiments I removed the head part and the stalk with the budding zone, and cut up the remaining abdominal parts into the smallest bits, which I carefully mixed among themselves, modelling them into a round mass of living substance. Out of a number of abdominal parts I always obtained one Hydra. But if there remained parts of the hypostome with tentacles, and parts of the budding zone, they grew into many-headed monsters—colonies of Hydras, reminding one of the well-known illustrations in Roesel-von-Rosenhof's works (1753). It is to some of the experiments made by this author that I owe the impulse to try some operations in the direction of dissociation.

When I mixed together finely cut up portions of the bodies of brown and of red Hydras and moulded them into lumps of this living substance, I obtained mosaic chimaeras by the *dissociation* method.

3. Lastly I took as model an experiment from the classical *Mémoire* of that pioneer of Experimental Zoology, Abraham Trembley (1744), and

repeated it with various modifications. This experiment may be termed one of *insertion*.

I chose a brown Hydra, and cutting off its stalk I pushed another entire brown Hydra, stalk downwards, right through the mutilated body so that the stalk protruded through the other end. I then fastened the two Hydras together with a pin, and left them in this position for a space of from four to eight or ten hours. The aim of this experiment was to unite the layers of two Hydras, but as a rule the two components would separate forming at the same time the most fantastic figures. In 1922, however, I succeeded in obtaining a true *association* during some of my experiments. Besides placing the animal organisms into a clearly determined position, I also attempted a reversed order: into the wide open



Text-fig. 2. Insertion-experiment. *a* = *P. oligactis* in abdominal part of body of *H. vulgaris*; *b* = *H. vulgaris* in *P. oligactis*.

mouth of the mutilated Hydra I placed another Hydra, head foremost, pushing it right through the body. Sometimes I left the budding zone untouched, and so on.

Believing that the want of success in my "insertion" experiments was owing to the fact of two heterogeneous walls touching—the ectoderm of the inserted organisms and the entoderm of the outer—I turned the Hydra which was destined for an inner component inside out, and placed it into the mouth of the outer one. This was effected in such a way that now two homogeneous surfaces touched, i.e. entoderm with entoderm (in a normal and in a reversed position). But the Hydras still continued to separate. I must say that a similar experiment has been already made by Trembley in 1744.

I used the method of insertion also for heteroplastic combinations, and carried out such experiments in 1922 on a large scale. Brown Hydras



were pushed into red ones and *vice versa*, in normal and reversed positions, turned inside out or left as they were (Text-fig. 2). The results were as follows:

At first I obtained *perichlinal* chimaeras (owing to the method of insertion) which, however, soon separated again, or turned into *mosaic* chimaeras.

But neither artificial sectorial chimaeras, nor mosaic ones, can exist long as such. In time regulating processes assert their power and bring about the total disappearance of the red substance from the surface of the body of the united Hydra. Yet a part of the elements of the red Hydra are retained in the body of the outwardly perfect preparation, especially the non-differentiated interstitial cells (the *I*-cells of P. Schulze 1918). I have therefore named the organism obtained as a result of these complex regulating processes, "*cytotoxic*" chimaera, a chimaera in which the cellular elements of the two parent organisms have become so thoroughly mixed, that they can no longer be separated into individual sectors, or mosaic, and so forth.

Further on I intend describing how I obtained natural sectorial chimaeras from Hydras, as well as the process of regulation in artificial sectorial chimaeras and those obtained by insertion; after which I shall speak at greater length about the multiplying process of cytotoxic chimaeras.

### 3. Natural Sectorial Chimaeras.

In the production of vegetable chimaeras of the sectorial type the following conditions are indispensable according to Baur:

In the growing cone there must lie on one side cells of one type (say white ones), and on the other of another type (for instance, green ones).

In Hydras we find a typical growing cone when buds are being formed. Now, if during a heteroplastic complantation-operation a brown bud-germ and a germ of the red Hydra accidentally meet along the common seam, they will, both becoming active at the same time, form a common cone of growth. The bud, which will grow from such a cone will be brown on one side, and red on the other. And according as the brown substance or the red preponderates in the formation of the chimeric bud, so will be its tint. Thus is produced in a natural way a typical sectorial chimaera which satisfies entirely every demand made upon it by Baur.

Instances: Exp. 118. Date 28. viii. 21. Complantation. *P. oligactis* + *H. vulgaris*. Three days after grafting appear rudiments of buds on

the seam; on 1. ix. four tentacles are seen on the bud; 2. ix. the bud is thrown off (Plate IX, fig. 1, *B.Ch.*).

Exp. 168. 28. vi. 22. Insertion of *P. oligactis* into *H. vulgaris*. 8. vii. Earliest appearance of bud; 11. vii. The bud is cast off with seven tentacles. It consists in even proportions of a brown and of a red substance (Pl. IX, figs. 2, 3, 4).

The natural sectorial buds were not distinguished by vitality. I was unable to carry them on to the budding process or to follow their further fate in succeeding generations. But in my opinion it cannot differ from that of artificial sectorial chimaeras.

In several cases there could be observed on the natural buds a more unequal and variegated distribution of the two colours, brown and red (Pl. IX, fig. 5).

In his experiments in 1922 Goetsch obtained similar sectorial chimaeras by grafting serially the green *H. attenuata* var. *viridescens*, on the top of the brown *H. vulgaris*. In my experiments buds appeared on the vertical and horizontal seam, while in Goetsch's on a horizontal one.

But these graftings are not so interesting, as both species *attenuata* and *vulgaris* belong to the same type of stalkless Hydras. Goetsch also succeeded in the serial union of the green *H. attenuata* on the top of the brown *P. oligactis*, but he did not notice any further propagation of these serial chimaeras<sup>1</sup>.

#### 4. Artificial Sectorial Chimaeras obtained as a Result of Complantation.

On August 14th, 1921, I found in Lake Parsolovskoye, near Old Peterhof, the red variety of *H. vulgaris*, and on August 19th I made the first heteroplastic experiments with it.

I cut off the stalk of a brown Hydra, and the lower part of the body of a red one. Upon this I opened the abdominal parts, unrolled them, and united the layers in the way described above (see p. 278, Text-fig. 1). When working at heteroplastic complantations I was obliged to leave the preparation pinned together a little longer, say two to three hours, as the free edges of the heterogeneous surfaces did not grow together easily. Still in most cases this adhesion did take place and the preparation used to assume a regular cylindrical shape.

<sup>1</sup> "Eine Vermehrung dieser Chimaeren habe ich noch nicht beobachtet." Information about Mr Goetsch's experiments upon serial grafting of Hydras was kindly communicated to me by him in a letter of the 15th of October, 1922, for which I wish to express to him my best thanks in this place.

In successful graftings a sectorial chimaera was at once obtained, consisting of two whole organisms. One half of the cylinder belonged to the red Hydra, the other to the brown one. A sharp limit was drawn between the two substances. The most interesting figures were presented by the hypostome. In successful cases there was formed a common mouth in its centre; one lip was red, the other brown. A sharply defined line divided the hypostome into two variegated hemispheres (Pl. IX, figs. 6, 7).

I must remind the reader that an important systematic sign which allows us to differentiate the red Hydra from the brown one (*Pelmato-hydra*) into a separate species, is the character of tentacles. In the red Hydra we find 6 to 8 (-10) thick short tentacles, while the brown Hydra has 6, as a rule (seldom more) thin, long, wavy tentacles.

In a chimerical preparation the character of the tentacles is completely preserved (Pl. IX, fig. 6): one edge of the hypostome is generally surrounded by a half-circle of short red tentacles, the other edge by a half-circle of long thin brown ones. In the beginning each group of tentacles gets shortened quite independently one of the other; but already two or three days after that, when the growing together of the fundamental layer takes place, a nervous connection evidently is formed and both groups of tentacles begin to contract synchronously.

In its later course the preparation behaves as if it were *one* organism, in spite of its consisting of two entire individuals which belong even to different species. This preparation responds to irritating influences, contracts, catches its prey, and swallows it, digesting it like any ordinary, only somewhat larger, Hydra.

But very soon after the operation, regulating forces become active in this chimerical preparation, which in an original manner entirely changes its outer aspect. In the year 1921 I carried out thirty-nine such graftings; in 1922 more than a hundred. In every case regulating processes interfered in the lives of these organisms, leading steadily up to the same result. I will illustrate these processes by a concrete example.

On the 19th of August 1921 I grafted two Hydras of different colours. A perfect fusion only took place in the central and lower portions, the heads of the Hydras did not grow together; each closed its hypostome and rounded off into a separate head; on each the tentacles shifted slightly to the other side. The boundary between the red and the brown matter was clearly visible on the abdominal part. The bottom part of the chimeric Hydra was composed of red matter as the tissues of the brown half terminated above the red (Pl. IX, fig. 8).

As early as four days later (23. viii.) a peculiar phenomenon occurred; the brown matter began as it were to spread over the red. The movement appeared first in the middle region of the abdominal part, and on the very next day the body of the red Hydra was seen to be girt round the middle as it were by the brown (Pl. IX, fig. 9).

The red head remained at the top, but a little below the brown one. The lower part of the red Hydra proved cut off; it turned into a foot, grew a sole which began to support the whole chimerical preparation.

On the edges of the red hypostome which were turned towards the brown head, there appeared two new tentacles. The increase in the number of tentacles is a common occurrence among Hydras whenever the interstices between the separate tentacles along the edge of the hypostome become too large.

All through this process there was no liberation of the red component out of the tissues of the brown one. Nor was there a spreading of the brown tissues across the red. A place in which there would have been four layers I was never able to observe. The boundary between the red and the brown substances was clearly visible all the time of settling. The regulation process itself consists in the disappearance of the colouring matter from the tissues of the red Hydra, and in the curious mixing of the cellular elements.

In the body of the animal organism there goes on as it were a regular fight between the tissues, resulting in the preponderance of the elements of the brown Hydra<sup>1</sup>. Outwardly the victory rests always with the brown Hydra; in reality, however, a part of the elements of the red Hydra remains in the body of the preparation, as I shall show further on.

Having seized the body of the red Hydra across the middle of the alimentary part the brown matter began to spread up and down—to the head and to the foot. Along the seam there still appeared traces of the red substance. The head of the red Hydra was already surrounded on all sides by the brown matter which, here and there, was even nearing the tentacles. At the same time the brown matter was spreading also over the foot (Pl. IX, fig. 10).

The next day, 8th day (27. viii.), a slight change took place among the tentacles: two brown ones united at their base and formed a bifurcation; eight red ones still remained of the same length. The lower portion of the preparation underwent a transformation: the abdominal part, which in the *H. vulgaris* typically narrows towards the base, changed into a

<sup>1</sup> The description of the histological processes will form the second part of my paper.

stalk characteristic of *P. oligactis*, containing however still traces of the red tissue. On the budding zone thus differentiated there appeared the first bud, in outward appearance the common *oligactis*, at first showing two, and then four tentacles.

On the following days the regulation process advanced at greater speed. The red head and the brown one approached each other; their hypostomes became almost fused into one common disc, and the crowns of the tentacles, the brown and the red one, began to pass one into the other (Pl. IX, fig. 11). The brown matter approached quite close to the bases of the red tentacles. The red hypostome, clearly distinguished by its colour, took up a position at the very end, so that the brown abdominal part seemed to terminate in two particoloured heads. But there was no struggle whatever between them about the food. The Hydra swallowed the *Daphnias* with its brown as well as its red mouth, which then passed on into the common stomach. No trace of the red substance was left in the stalk.

On the 3rd of September (the 15th day) there were to be seen on the common hypostome five brown tentacles (two having accomplished their junction) and seven red ones; one of the latter showed bifurcation.

On the 4th of September there appeared two buds at a time—their outward appearance (all the tentacles folded themselves up simultaneously) being typical of the *H. vulgaris* (Pl. IX, fig. 12).

The budding process proved the good condition of the specimen under examination, which fed and grew excellently all the time. Its general look was now that of the typical brown Hydra (*oligactis*), with a clearly definable stalk and a budding zone. (Of the latter I shall speak further on.)

The subsequent regulating processes were centred in the still complex hypostome. The brown substance moving under the base of the red tentacles seemed, as it were, to spread and pass under them and to cut them off from the disc near the mouth which belonged to them. The upper end of the Hydra was now crowned by one hypostome surrounded by a wreath of twelve tentacles (five brown and seven red ones); between the tentacles there were two mouths, the one brown, the other within a circle of red matter. But in a few days the two mouths joined, and the red substance was pushed to one edge of the hypostome, resembling a small sharply defined island.

But having approached the base of the tentacles, the brown substance did not stop there; it began to creep up the very tentacles. On the 19th day after the operation (September 7th) the common hypostome

presented a very unusual and quite unique appearance. All the red tentacles had been pushed away from the edge of the brown hypostome to a considerable distance (Pl. IX, figs. 13 and 14).

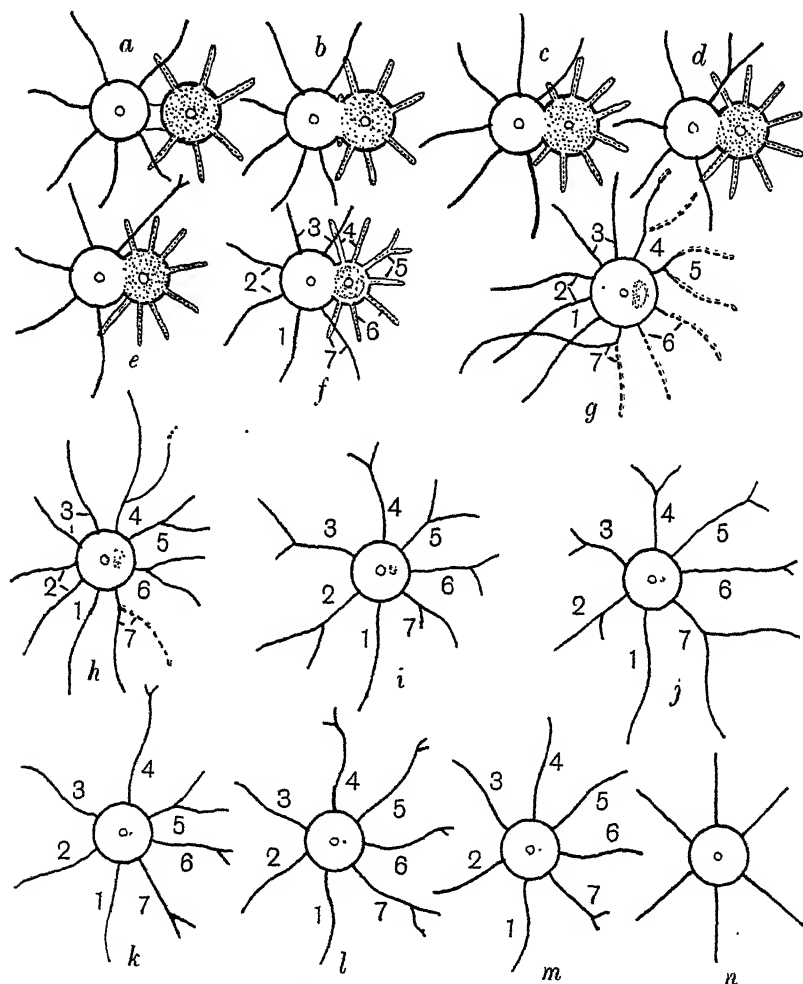
It must not be forgotten that the tentacles of the Hydra possess the faculty of constant growth, and in proportion as their ends are worn out they are replenished from their base near the hypostome. Not infrequently do we notice bifurcations on the tentacles; the causes of their appearance being very manifold. They are generally explained as examples of the splitting of the tentacles (which, as far as my experience goes, hardly ever happens); or as an example of the junction of tentacles (which does happen, but not often); the most frequent explanation of the phenomenon, however, being that the bifurcation is slowly pushed upwards from the basis of the tentacle to its tip, and that the diminution of the branches is due to the uninterrupted growth of the tentacle. The absence of union and the fact of uninterrupted movement have been mentioned by Boecker in 1914 in connection with the following experiment (made also by him). In a bifurcation consisting of two equal branches I kept cutting down one branch to one-half; this bifurcated tentacle continued after that to move away from the hypostome and to dwindle down at the tips—but the relative proportion in the size of the two branches remained the same after the operation to the very end.

After these introductory remarks, the evolution of the processes which take place upon the hypostome grow more comprehensible. A series of diagrams represents the consecutive growth of the regulatory process. It must, however, be borne in mind, that it is exceedingly difficult to identify the separate tentacles owing to the rapidity of the regulation process, especially if more than twenty-four hours pass between the examinations. The figures placed near the consecutive stages of the development of the Hydra, can therefore lay no claim to absolute exactness (Text-fig. 3).

The figures 1–7 correspond to the final number of tentacles that have grown up in place of the old (regulated) ones. Figures 1–3 mark the five old tentacles of the original *oligactis*. Figures 4–6 the six red tentacles of *vulgaris*. Figure 7 represents a pair of tentacles grown out of the seventh red tentacle and the sixth brown one (which appeared on September 3rd).

I shall not enter into an explanation on the causes of the numerical (regulation) change of tentacles and of their decrease from twelve to the normal number typical for the species *oligactis*, i.e. six in spring and summer (six) to seven or eight in late summer and autumn. This is partly owing to the growing together of the separate tentacles in pairs,

and partly to the uniting of those originating centres of their growth which we place at the edge of the hypostome, below the visible base of the tentacles. But possibly also because of the appearance of new germinating centres between the bases of two old tentacles. In these two last cases the new tentacle converts its two adjacent old neighbours



Text-fig. 3. A scheme of the regulation of tentacles on the hypostome of the sectorial chimaera (Exp. 105, 20. viii. 21). Diag. *a* = 21. viii.; *b* = appearance of two new red tentacles (27. viii.); *c* = 26. viii.; *d* = fusion of two brown tentacles (27. viii.); *e* = 31. viii.; *f* = fusion of two red (5) tentacles (3. ix.); *g* = fusion of three pairs of tentacles—7 (red and brown), 5 (red and red), 7 (red and brown), 7. ix.; *h* = 8. ix.; *i* = six pairs of regulating tentacles (2-7), 10. ix.; *j* = 11. ix.; *k* = 12. ix.; *l* = 14. ix.; *m* = 15. ix.; *n* = scheme of a normal six-tentacled Hydra.

into a bifurcated tentacle which may even consist of the tentacles of two species of *Hydra* (cf. diag. *g*).

Two red tentacles (marked by the figure 6) were simply pushed aside by two growing roots of brown tentacles. Two others—5—(diag. *g*) were pushed out of place by one common brown tentacle which had grown up under their bases during the regulating process. The other red ones were driven still further out of place. One of them remained in a fair condition, whereas the other which had been pushed to the very edge, had its series of nematocysts closely packed; the whole tentacle had been kneaded into one small red head (diag. *g*, 4). The last of the red tentacles (marked by the figure 7) had been simply seized by the overgrowing brown tentacle and carried outside the hypostome.

During the following days the regulation process extended to the tentacles of *oligactis* (diag. *h* and *i*). Four free tentacles (2 and 3) became united in couples (were pushed outside by their common bases), having formed characteristic bifurcations. In the red half of this section the union of the red tentacles proceeded its own way.

On the 10th of September (the 22nd day) the hypostome made an exceedingly beautiful picture (diag. *i*). Out of seven tentacles six were bifurcated. The disc of the hypostome was entirely brown, with the exception of a small red spot between the mouth and the basis of tentacles. The bifurcated ends of the tentacles showed but faint traces of the red matter which soon disappeared entirely. There remained an impression that while the red tips were shrinking together, the substance of the brown tentacles was spreading to the bifurcations themselves.

On the 24th day (September 12th) three tentacles had been entirely regulated; on four of them one could still perceive bifurcations (diag. *k*). On the following day the bifurcations had travelled down to the very tips of the tentacles, and on the next day there was only one bifurcation left; and at last, on the 28th day (September 16th) there sat in the vessel a normal *Hydra* with seven tentacles to all outward appearance a perfect *oligactis* (diag. *m*). Only a faint red spot on the hypostome reminded the observer of the vanished red component part. On the 20th of September the preparation perished.

But the budding process of the *Hydra* had been unusual (it had produced only nine buds); and this made us suppose at once that the original brown *Hydra* had undergone profound changes.

Beneath the typical appearance of *oligactis*, which had returned to it as the result of the regulatory process, was concealed a very unusual nature. The even brown colouring was hiding the presence of cell-elements



which had remained in the body of the brown Hydra after the union of its tissues with those of the red Hydra and especially with its non-differentiated, interstitial cells (*I*-cells after P. Schulze 1918) which, together with the non-differentiated cells of the brown Hydra, had evidently formed a strange, not compact, mixed layer of the cells of both species. These cells had proved capable of living side by side in a strange manner, keeping their genotypical purity. All this became clear during the process of budding, which is explained farther on. Therefore we consider the organism which appeared as the result of the regulatory processes, to be a true typical chimaera, which consists however, not of two genotypically different, united tissues, but is the result of the mixture of loosely bound cells. For this phenomenon of the mixture of cells I would suggest the name of *cytomixis*. Therefore the chimaera which appeared as the result of this mixture might conveniently be called a cytomictical one.

The next section of this Essay will demonstrate the course of the regulating processes as shown in three "insertion" experiments of widely different outward changes of form, but uniform in their inner modifications and final results.

#### 5. *Chimaeras as Results of Insertion.*

It is a characteristic of experiments in insertion, as Trembley has observed, that the component parts show a tendency to separate.

The aspects observed in such cases are exceptionally varied even during simple homoplastic operations. The regulatory processes become still more varied in heteroplastic combinations, so that it would be right, strictly speaking, to describe every individual experiment separately, if we paid attention, principally, to the outer changes during the course of the process.

The stage of life of the periclinal chimaera continues but a very short time. The tendency to part manifests itself already on the day after the operation. This breaking asunder however, is far from being always accomplished smoothly. The components have, on the contrary, to pay for their freedom with parts of their bodies. These pieces are being drawn into the sides of the body of the liberated component part and form there either a chimerical section or mosaic-like portions which are absorbed during the following stages of the regulatory processes.

Of far greater interest are the cases where the union of the components becomes a more lasting one. I shall describe in detail one such experiment (164) which was the cause of my genetical investigations of the generating processes of animal chimaeras.

On June 28th, 1922, I inserted a rather big six-tentacled red Hydra into a narrower brown one (see Text-fig. 2). On the next day the preparation was in excellent condition; the components had already begun their parting. The head of the brown Hydra had sunk to one side while crawling down from the red one. The whole parting process was following a peculiar line, which I observed also in some other cases. The tentacles which had at first surrounded the hypostome of the Hydra, which was being greatly stretched by the body of the red Hydra in it, now began to move to one side as it were. The substance of the hypostome followed the movement of tentacles and began to shape itself into a new head. The body of the red Hydra meanwhile cut a passage through the side wall of the brown outer Hydra and appeared outside. During this process the walls of the brown Hydra kept closing behind each fresh piece of the red Hydra that passed through it (Pl. X, figs. 15 and 16, viewed from the side and the top on the 1st and 2nd day after the operation).

On the fourth day (July 2nd) it was clear that the brown Hydra had freed itself entirely from its inner component, but in doing so it had torn the latter in half (Pl. X, figs. 17 and 18). The rupture had taken place where (on the abdominal side of the preparation) a ring of brown matter had remained longest on the abdominal region of the red Hydra. The remarkable appearance of the preparation deserves attention. On a ridge of the hypostome of the brown Hydra there rose on one side of it a crest of six long tentacles; in front of these there appeared a new mouth. The body of the Hydra was inclined to one side, towards what had been before the "abdominal" part, to which the two halves of the red Hydra had become attached. From the upper part there stretched a piece of red substance to the hypostome of the brown Hydra. The lower half of the red Hydra had formed the "abdominal" wall of the whole preparation. The red foot with its intact sole served to attach the preparation to its substratum. (The brown Hydra had had its stalk and sole removed before the operation.) Pl. X, figs. 17 and 18, represent the preparation from the side and back.

On the following, fifth, day (July 3rd) the Hydra, not being well looked after, ate some Daphnias (I generally begin to feed the heteroplastic Hydras somewhat later); but this mishap gave us the possibility of defining more accurately the limits to which the red substance had spread in the body of the brown Hydra (Pl. X, fig. 19). The upper and the lower red sections proved to be entirely severed; only a narrow stripe showed the former union. The head and the upper part of the abdominal portion of the red Hydra had become entirely separated; taking up a position

at right angles to the brown Hydra, the red one looked like a monstrous "right arm"; the cavities of both Hydras had become united (Pl. X, fig. 19).

But most interesting of all was the head end of the brown Hydra. We noticed that on this protuberant hypostome there had just appeared a new mouth and that the former six tentacles had taken up their position along one edge in the shape of a straight crest. The opposite section of the circle was empty. Such a relation cannot exist long in Hydras. As soon as the spaces between the tentacles on the edge of the hypostome become too wide (the causes are various—a tear, a cut, rapid growth, a push, a move aside, or any other reason), there appear immediately new growths of tentacles which fill up the interstices. From this point of view the hypostome of the Hydra might be called a kind of "organizing centre" whence issue stimulant form-determining influences, which call forth definite structures—in the present case—tentacles.

What the nature of these stimulant influences may be which form a condition of such new developments, we do not undertake to decide in this place. It may be of a transcendental or physico-chemical character, or it is possible that in this process the so-called hypothetical "morphohormons" take part. The idea of organizing centres that act during new growths is to be found in Spemann's work (1921) and that about "morphohormons" in Zavodovsky's book (1922).

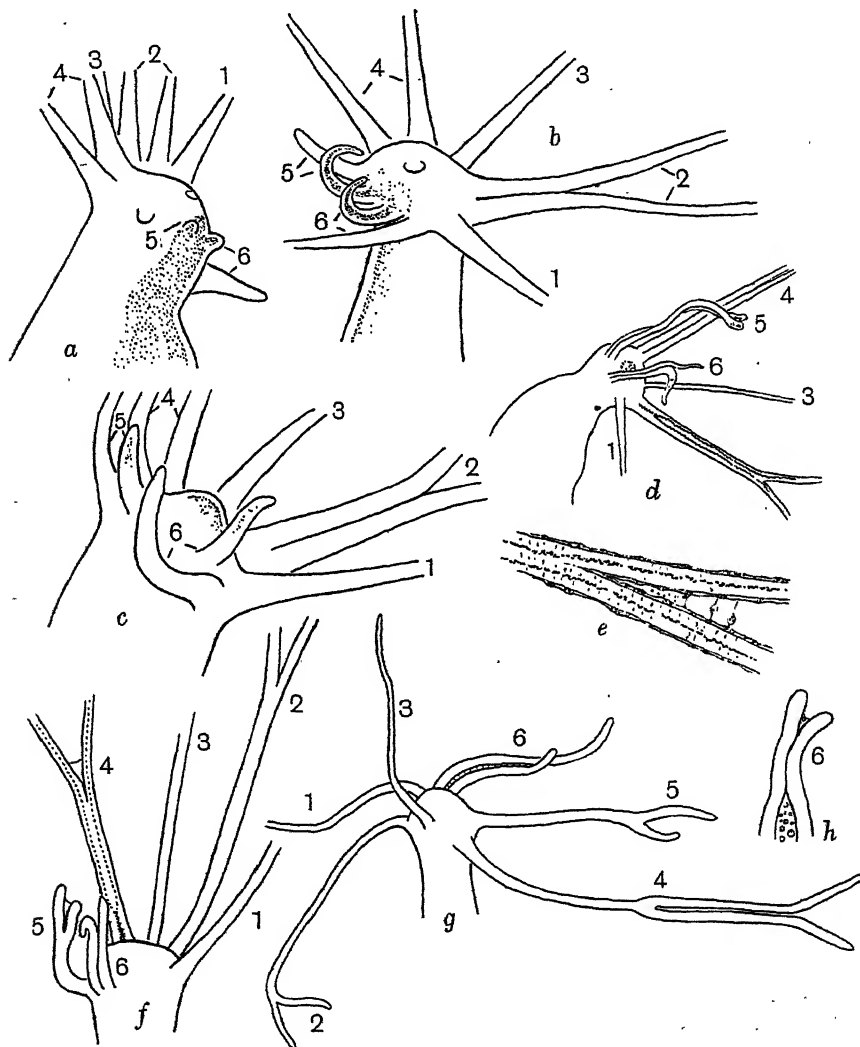
However this may be, on the free edge of the hypostome there had shown themselves the beginnings of four new tentacles. Of these two were brown and *two red*. A piece of the red substance fell accidentally on the edge of the brown hypostome during the operation, i.e. within the zone of its form-determining influence, or to follow Spemann, into the "field" of the organizing centre, defined by the edge of the hypostome—and these irritating influences transformed the alien tissues so as to create a single harmonious whole.

During one of the Bierens-de-Haan's experiments there were found on the chimerical larva of a sea-urchin *Parachinus microtuberculatus* in a section composed of the tissues of the *Paracentrotus lividus*, cells with big ciliae, indispensable for bringing forth a whole ciliary cord, which had accidentally run along this section during the process of its formation.

In Spemann's experiments the edge of the blastopore of the developing egg of *Triton taeniatus* which had been placed into a hollow of the egg surface of *T. cristatus*, engendered in the foreign mass the same kind of medulla-plate as it generally forms in its own substance.

These facts have a deeper meaning. They tell us that the potential possibilities (Driesch's prospective potency) of certain cells are greater than their realized ones (prospective value).

The cells containing red matter, which had gone to build up new tentacles, belonged to the abdominal section. During the regulation process, however, they acquired a new destination.



Text-fig. 4. Regulation of the number of tentacles on the head of chimerical preparation (Exper. 164). Diag. *a* = 3. vii. 22; *b* = 5. vii.; *c* = 7. vii.; *d, e* = 8. vii.; *f* = 10. vii.; *g, h*, = 11. vii. For explanation see text.

In my above-mentioned experiment I paid special attention to the regulation-process as it took hold of the hypostome and its surrounding tentacles. Thanks to this, and to my marking the changes in the hypostome day by day, I was able to follow the vicissitudes in the life of each tentacle. Therefore also, the diagrams printed further on, are fuller than the rough sketches of the experiments in 1921, and illustrate the rapid succession of changing events in the lives of Hydras.

The ten tentacles that enclosed the hypostome on June 28th, became regulated down, within twenty days (by July 17th), to their normal summer number, six, which is typical for the species of *oligactis*. Having given the name of First (1st) to one of the tentacles that remained unchanged to the very end of the "regulation," I shall mark the rest of the tentacles by corresponding figures (Text-fig. 4 *a*). 1 = the first in the row of the old tentacles of the fundamental brown Hydra. The next two old tentacles made one, the second (2nd) of the future Hydra. The fourth tentacle became by and by the 3rd; the fifth and the sixth gave the fourth (4th). The new brown tentacle and one of the red ones made the fifth (5th) tentacle; another heterogeneous couple formed the sixth (6th) tentacle of the future "regulated" Hydra.

The series of drawings illustrates these processes (Text-fig. 4).

I return to the broken off thread of my account of the stage of development investigated by me on July 5th, i.e. on the seventh day after the operation (Text-fig. 4 *b*).

A couple of brown tentacles were beginning to unite (2). The red tentacles had grown; but two days later they had already grown considerably paler, browner, their red substance appeared to be concentrated at their very tips (diag. *c*). All this pointed to the fact that the hypostome had begun to bring forth a replacing growth of brown tentacles on the very spots where the red tentacles had sprung up.

Diagram *d* (July 8th, the 10th day) shows the condition of the Hydra shortly before the total disappearance of the red matter. Two tentacles, corresponding to the future 5th, had become intertwined; one of them bore a pin's point of as it were coagulated red matter on the tip. Two others (the future 6th tentacle) had commenced the process of uniting. The "regulation" had seized even the old tentacles. Already the day before, two of the tentacles (the future 2nd) had approached each other very closely, and now the seam which had separated them had disappeared from the base of the tentacles. Here the fusion of the tentacles was complete, which fact was supported by the evident union of the

entodermal canals. Farther down the tentacle these canals ran side by side in two parallel lines, and only at the very end both tentacles and canals parted, forming a bifurcation (diag. *e*). We can easily conceive that the tentacles were joined first in their outer surfaces, after that their ectodermal membranes became united, and lastly their entodermal canals (diag. *f*).

Two other old tentacles had lain down side by side, parallel, in order to form the future 7th tentacle.

The following drawing (diag. *g*) represents the process of regulation in full vigour (July 11th, the 13th day). Of six tentacles four had already forks. The future 2nd tentacle had pushed its bifurcation nearly to the utmost tip. The future 7th tentacle had carried its two branches, which on the previous day had been lying stretched out side by side, far away from the hypostome, together with the thin web that united them. The 5th tentacle shared the same fate. But the regulation of the 6th tentacle proceeded with far greater difficulty. In spite of the growth of the membrane between them, the two tentacles found it for a long time very hard to become fused (diag. *h*).

Text-fig. 5, diag. *a* (July 13th, the 15th day) represents a three-branched tentacle that appeared unexpectedly, and corresponded to the future 4th tentacle. A comparison with the preceding drawing (diag. *b*) shows that in the spot marked thus ↓, there had evidently taken place a juncture of two forks lying side by side. After this there must have occurred a rupture to the left of this spot which freed the base of the adhering tentacle while the tip was seen to be "transplanted" to another tentacle. (This is the phenomenon of autotransplantation of tentacles, which I have had occasion to observe also during other experiments.) The regulation of the other tentacles needs no further explanation.

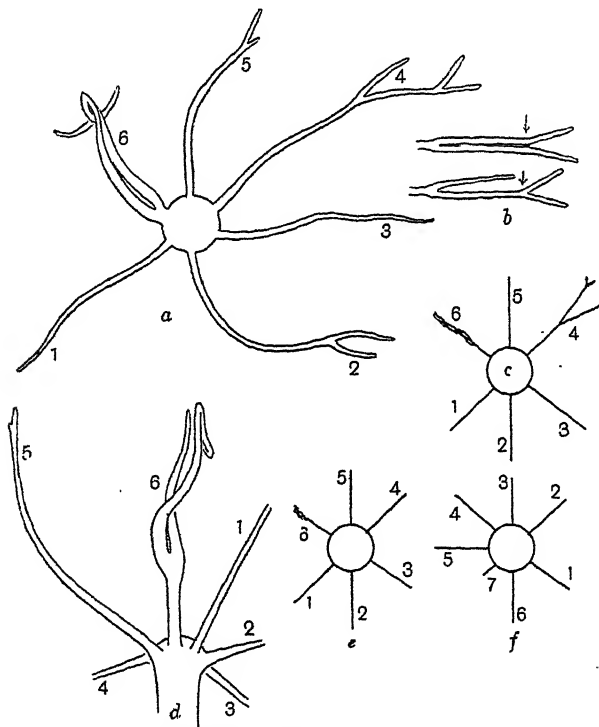
The greatest difficulty in regulation was experienced with regard to the couple of tentacles that furnished the 6th future tentacle. Both kept growing side by side evenly, without uniting. But at last there was formed a common new base under them, which pushed them away from the hypostome (Text-fig. 5 diag. *c*).

On July 15th (the 17th day) the process made a great stride forward (diag. *d*). Besides this 6th tentacle, only the 5th showed still signs of bifurcation, but only at the very tip. At last the two branches of the 6th tentacle became intertwined in a ball which continued to move away from the hypostome, at the same time dwindling down at the end.

On July 17th (the 19th day) the Hydra possessed six tentacles (diag. *e*) and between the 5th and the 6th there had appeared the begin-

ning of a new tentacle, the 7th (diag. *f*). The number of these latter increased to eight, after which it decreased to seven, and then to six (on the 19th of August). The Hydra ended its life as a normal, six-tentacled *oligactis*, 65 days after the operation.

We shall now be able to answer the question about the "fusion" of tentacles and to allot the bifurcated tentacles their right place in the regulation process.



Text-fig. 5. The same, continued. Diag. *a*, *b*=13. vii.; *c*=14. vii.; *d*=15. vii.; *e*=17. vii.; *f*=20. vii. Explanation in text.

The diminution in the number of tentacles is brought about in a two-fold manner.

(1) The tentacles are brought together lengthwise, when webs will occasionally be produced, or fusions, etc. In this case the united parts are thicker than a normal tentacle. This process may spread to all the tentacles, but it is frequently confined to a part of them only. This united portion is then pushed out by a new tentacle which grows up out of its base; or (2) there is formed a new growth on the edge of the

hypostome between the bases of the two tentacles, which growth immediately pushes out the two old ones in the form of a bifurcation.

I shall now return for a while to the red component and its fate, and sketch the changes which it underwent in its separate parts.

The *red tentacles* disappeared very quickly from the brown head of the fundamental Hydra. They appeared on July 3rd and grew very slowly at first, owing no doubt to the scanty amount of nourishing red matter on the edge of the hypostome. As pure red tentacles they existed only three days; after which the red pigment began to disappear out of them, and they were carried away from the hypostome by the brown tentacles which had grown up under their bases. A few days later, on July the 9th, the last traces of the red element vanished entirely (Text-fig. 4, diags. *a-d*).

The *red portion of the hypostome* diminished in size very fast, centering at last in a rather small red spot. Later on the red matter was driven away by the brown substance, from its position on the edge of the hypostome nearer to the aperture of the mouth, after which it disappeared entirely together with the red substance in the tentacles (Text-fig. 4, diags. *a-d*).

The *red section in the walls* of the body disappeared faster still. On July 3rd they were still clearly visible, but only two days later the lower part of the stomach had grown quite brown, while in the upper part (Pl. X, fig. 15) the red substance extended from the brown hypostome to the base of the red Hydra in the shape of a narrow, in some places torn, ribbon; and two days later still, every trace of the coloured elements of the red Hydra had disappeared from the body of the brown Hydra.

The interchange between the *red foot and sole* and the cells of the brown Hydra occupied a comparatively long time. We observed (p. 289) that the red foot was at first placed on the "right" side of the chimerical preparation. It then shifted to the bottom and took up a terminal position. As the final result of some rather complicated regulating and reducing processes, we found a considerable diminution of the red portion of the common foot or base. For a little while longer I was able to discover red elements in this common foot—that was when the foot itself had already assumed its perfect form and had turned into a typical "*oligactis*" stalk. Sixteen days after the operation, however, after July 13th, the last traces of the red matter had become eliminated from this stalk.

The fate of the liberated *abdominal portion* of the red Hydra was as follows: On the 3rd of July, five days after the operation, the separation



of this portion took place, but for its absolute completion we had to wait about a month. The head end of the red Hydra remained all this time without change. It kept its bright red colouring to the end, as well as the same number of tentacles (viz. six). In the spot where the stomach portion touched the body of the brown Hydra, there seemed evidently a constant struggle to be going on between the cellular elements, which found its expression in the change of colouring of this portion (Pl. XV, fig. 47).

From red it changed first to a deep orange brown, then to brown with a slight reddish tint. During the period when the fundamental component was forming the stalk and thus liberating into a separate part the budding zone, the red Hydra was lying on its body in the shape of an isolated bud which was placed above the budding zone (10th-11th days). On the 10th of July (the 13th day) it had already reached the budding zone (Pl. XV, fig. 48).

It must be remarked, that in those experiments on transplantation, when the added component is lying at the side and below the head, it usually advances by degrees towards the budding zone along the abdominal section. This is the critical moment for this alien component part. If by this time it has managed to develop a true stalk or foot, the original Hydra throws it off in the form of a bud. But if for some reason or other it is retarded in its development, then the budding zone passes it by, as it were, rising above it, while the alien component remains fixed to the stalk and slowly descends along it to its very sole. Here, at last, the final separation of the components takes place. The above-mentioned stages in these processes had made it possible to establish the fact of "longitudinal division," which does not seem to be proper to Hydras.

The same fate overtook the red component during my experiment. It had not been able to develop a true foot when the brown budding zone began to pass along it (Pl. XV, figs. 49, 50), notwithstanding its having thrown out a real bud by July the 9th. (The species *vulgaris* does not really possess a stalk, which explains the want of it in this case.) On the 13th of July the red Hydra was below all the buds of the brown one, but still on the border of the budding zone, but two days later there was already a remarkable distance between the foot of the red Hydra and the budding zone of the brown one (Pl. XV, fig. 52). Evidently the direct communication between the alimentary canals of the two components had come to an end.

On the 11th of July there had appeared two testes under the head of the red Hydra; three days later we became aware of a trembling motion

among the developed spermatozooids. In the lower part of the abdominal region first one egg developed, then another (spec. *vulgaris*—bisexual; *oligactis*—with sexual organs on separate individuals). These eggs became immediately fertilized (during the process of self-fertilization); covered themselves with a spiny membrane characteristic of *vulgaris*, and were freed (Pl. XV, fig. 52).

I was interested in ascertaining whether the change to a sexual state might not influence the relations between the two component parts. Might it not bring about a sexual process in the brown Hydra likewise? If this had been the case, we might have spoken of the presence of special sexual hormones even in Hydras, which could act at a distance. But the development of testes and eggs in *vulgaris* did not influence the soma of the brown Hydra in the least.

About the 20th of July the red Hydra was sitting in the middle of the brown Hydra's stalk; by the 27th it had been pushed nearer to the base, and on the 27th, that is, a month after the operation, it was cast off in the shape of a rather small Hydra, which had become very brownish, but bearing still a bright red hypostome. I did not carry on my observations of this Hydra, as it was evidently greatly enfeebled by the sexual process, with but little vitality, and indeed soon perished.

I have refrained till now from touching the fate of the fundamental brown Hydra, which had budded plentifully while under observation. Within two months it had grown 65 buds in all. I have kept silent intentionally, as I shall have to speak in detail of the vicissitudes of this ancestral brown Hydra when describing the budding process.

#### 6. *Another Instance of an Insertion Chimaera.*

I shall now describe the fate of another chimerical preparation, which was the result of the insertion method of treatment. For this experiment (Exp. 168) the brown Hydra was placed inside the red one. The experiment was carried out by me on the 28th of June 1922. Next day the preparation still bore the appearance of a transitory, periclinal chimaera (Pl. XI, fig. 20), but on the following day the aspect changed abruptly (Pl. XI, fig. 21).

The inner brown component split in two. The upper portion of the abdominal section, together with the head and tentacles, crawled out through the mouth of the red Hydra, but still remained in contact with it. The edges of the red mouth grew together with the lower edge of the torn-off brown abdominal part.

The lower half of the brown Hydra also crawled out, together with

its stalk, through the lower aperture of the red Hydra, but also remained united to it. The whole preparation had a very strange aspect. In the vessel sat a Hydra with a two-coloured abdominal section—its lower part was brown, the middle red, and the upper portion again brown. The common abdomen filled all the three portions.

This method of parting asunder was not new to me, as I had observed it already in the year 1921 in homoplastic insertions.

On July 2nd, the fourth day after the operation, there appeared a new mouth on the border between the brown and the red portions of the abdominal section, surrounded by a crown of red tentacles. It was formed at the side, in the red substance, between the red tentacles which it slightly pushed aside during its development.

At the same time there grew up out of the brown substance the first bud (*B1*), within the budding zone at the lower end of the preparation. This, too, was not unexpected, as the *oligactis* which I had used for this operation, had preserved its budding zone. The lower border between the brown and the red parts was clearly and sharply defined. The brown substance surrounded the red, like a cup.

The next day brought an unexpected change. The place where the regulating process was progressing during the experiment was the upper border between the red and the brown abdominal portions.

Only on the previous day there had appeared a new mouth in this spot. In what way it had been formed—whether during a process of new formations, or as the result of the moving aside of the edges of the old mouth—I shall not undertake to say—besides it hardly matters much. Only on the day in question it had produced around itself a kind of “organization field.” The red substance which was on one side of the mouth aperture had turned into a half-disc of the hypostome; while four of the six red tentacles had already placed themselves along its edge, having moved over here from the place they had occupied before around the brown component.

It is difficult to give reasons at present for this transmigration of tentacles and for other phenomena which are connected with the plasticity of living matter. This same quality manifests itself also in the shifting of grafted component parts along the body of the animal; when a Hydra which has been turned inside out, turns in again; or when the wall of an organism closes again after one of the components has passed through it; and such like.

In the Hydra this plasticity approaches the fluidity of such solid bodies as ice, wax, boiled resin, etc. We shall have to look upon all these

movements on the one hand as manifestations of processes accompanying growth and on the other we shall have to look for an explanation to the laws of surface tension of colloidal matters, and to the consequences of attempts to regulate that tension which probably arises during heteroplastic junctions.

But the organizing influence of the newly created red hypostome was not limited to the transplanting of the red tentacles to one side. It brought within its sphere of action the bit of brown substance lying immediately next to it, and "organized" it also into the missing half of the hypostome.

This took place as follows: Out of the adjoining walls of the brown Hydra there was put out first one tentacle (Pl. XI, fig. 22), and then two more (Pl. XI, fig. 23). At the same time, the longitudinal part of the brown Hydra was bent from its original position to an angle of about 60 degrees.

I must point here to two important moments. In the first place, we must turn our attention to the change of polarity in the abdominal section of the brown Hydra. To build up new tentacles, i.e. a new head part, a portion of the body was used which, under ordinary circumstances, would have remained in the lower, aboral, part of the abdominal wall. This transformation compels us to reckon the newly formed head among the typical polar heteromorphoses.

The fact itself, however, of the appearance of a new head makes us consider this heteromorphic formation as the result of the manifestation of latent potential possibilities of interstitial cells, evidently provided with the same totipotentiality, as the fertilized eggs. In this special case we are even able to point out the irritating cause, viz. the hypostome, which called forth this heteromorphic new formation.

In the second place, it is necessary to note that the organizing influence of the hypostome makes itself felt equally strongly in both species. The hypostome, also a kind of "organizing centre," draws forth new tentacles out of every substance that happens to fall within its sphere of influence. In the foregoing experiment we saw how the brown hypostome drew forth new tentacles out of the red substance; in this case we noticed how the red substance built tentacles out of the parts of the brown hypostome that collided with it.

I return to my interrupted description of the regulating processes.

The following days brought comparatively few changes. The new brown tentacles continued growing in length; the new mouth aperture

with its crown of tentacles began to mould itself into a regular head; and simultaneously with this, the upper brown portion of the abdominal section grew considerably—the preparation was, in point of fact, feeding well at that time; besides it was all the time declining from its original axial orientation and was bending towards its stalk.

At the moment of operation the red Hydra had passed into the sexual state. The testes had appeared, the ovaries were established, but these organs advanced very slowly and by degrees diminished in size. In spite of the continuance of the sexual process, the tissues of the red Hydra were drawn into the budding process; they were forming typical sectorial chimerical buds, which originated in the budding zone that lay beneath the brown Hydra, as shown in the affixed illustration (Pl. XI, figs. 24, 25, 26).

The same illustration shows also the changes which had come about by the ninth day after the operation, on July 7th. First of all we observed that the upper abdominal part of the brown Hydra, which had considerably increased in size, had bent down towards the stalk taking up a position at right angles to it. This bending had taken place under the influence of the new head. The latter had taken up a correct axial orientation with regard to the stalk and the base. There were probably issued from the base special form-determining stimuli which turned the new head part that had arisen in the *side* wall, into a morphological *upper* end. In consequence of this the abdominal section of the brown Hydra settled mechanically down lower. Physiologically, both mouth openings carried out their functions similarly. Only the new mouth did so at first less successfully.

Turning our attention to the new head that was formed at the point of the bend, we shall find it has a typically chimerical structure (Pl. XI, figs. 25, 26). One-half of the hypostome bears six red tentacles, the other half three brown ones. The mouth is surrounded by both substances. Besides this we shall see that the brown substance seems to have begun taking a more active part in the building of the complicated head part (as if it were moving on to its red component). I remind the reader here of the fact that the forming of the new chimerical head was called forth by the red tentacles and the red hypostome. In my Diary of Observations I made the following note on that day: "I should not wonder if they had to pay with their lives for this game!"

This prophecy was but too soon to come true.

A space of only three days divides the stage depicted in Fig. 24 from that of Pl. XI, fig. 27 (July 10th). The abdominal part of the upper brown Hydra fed very well all the time, and grew unusually; this was

especially noticeable in the aspect it presented on the following day (Pl. XI, fig. 30, July 11th). The new head, on the contrary, stopped taking in food.

This stoppage was due to the regulating processes which were beginning to make themselves felt. The brown substance began its attack upon the red half of the hypostome (Pl. XI, fig. 28) and was rapidly cutting it off from the red abdominal part lying below (Pl. XI, fig. 31). The red tentacles, having no longer any food reserves (*I*-cells) of their own kind, stopped growing (Pl. XI, fig. 31), while under some of them the formation of brown bases could already be observed.

Not unfrequently have the regulating processes a depressing influence upon the organism which, in the case of *Hydras*, is accompanied by an increase in the stickiness of the surface layers of the body (as Boecker has shown in 1914). Probably as a result of this one of the tentacles assumed a grotesquely bent shape which was afterwards also regulated (Pl. XI, fig. 29).

The abdominal part of the red *Hydra* was divided from the substituting brown substance by a pretty clearly defined line. At the same time it grew, however, much browner, becoming really brown, with a slightly reddish tint.

The chimerical bud, which had grown up in the brown budding zone had already regulated its red section, and had become entirely brown. The strange union with the maternal organism is worth attending to. It was joined to it not by the stalk end, but by its side wall. This was to be foreseen, as shown in Pl. XI, fig. 27, by its condition at that time. Above it grew up a second sectorial chimerical bud (Pl. IX, figs. 2, 3, 4). Both were cast off on July 11th (Pl. XI, fig. 27).

I have already mentioned the unusual growth of the brown *Hydra*'s abdominal region. A look at Pl. XII, fig. 32 (magnified precisely in the same scale as all the foregoing drawings) shows to what extraordinary dimensions the organism had grown. This was on July 14th, the sixteenth day after the operation. But at the same time there appeared a corrective suited to that growth.

In my other grafting experiments on *Hydras* I had observed true polar heteromorphoses of characteristic structure. The alimentary canal of the abdominal region possessed at its two opposite ends two mouths and two wreaths of tentacles. Such heteromorphoses became regulated in a very odd manner. They grew a tube of unusual length which, instead of breaking in two in the middle, developed in the middle of the abdominal section, a budding zone. In order to form stalks the substance

of the abdominal section had to de-activate itself; and this was reached by means of budding. One after the other pieces of live matter were detached to form buds, and thrown off the preparation. In the inactive zone thus obtained, there then took place a bend; a common base was formed, after which the two Hydras parted company.

The same thing happened also in this experiment. The abdominal portion assumed disproportionate dimensions. But as the regulation of proportions has evidently only definite means at its disposal, no splitting took place; in the middle of the abdomen, nearer to the chimerical head, there arose a budding zone. (Evidently the distance between the head and the budding zone is proportional to the dimensions of the head part.) From this budding zone the de-activations of the abdominal region proceeded along two lines: towards the brown head, and towards the chimerical head. Towards the latter four buds (*B'* 1-4) were drawn; to the former three buds (*B'* 5-7). It was difficult to determine the orientation of the eighth bud (*B'* 8); probably a portion of live matter which had remained in the budding zone had been made use of for its construction.

In the chimerical head the regulation process was going on at full speed (Pl. XII, fig. 33). From the red hypostome only a small portion was left in the region of the mouth. All the red tentacles were carried off by the brown tentacles, which had grown up in their bases. The red pigment remained only in the terminal sections which were being glued together. In the place of three brown tentacles and six red ones, nine brown ones surrounded the hypostome which had grown up meanwhile, and were already beginning the regulating union.

The abdominal part of the red Hydra had grown almost brown, yet the line of demarcation where the substitution of the brown element for the red had taken place, could still be traced slightly (Pl. XII, fig. 33).

In spite of the regulation process, there were still traces of the testes, there was even an attempt made on July 17th to develop an egg, which, however, was not fertilized, and was absorbed.

On the 21st of July the preparation suffered from depression (Pl. XII, fig. 34). The budding process stopped after having thrown off about ten buds. The process of regulation in the head part continued active. The remainder of the red entoderm in the abdomen, above the common stalk, individuated very sharply (a phenomenon which I did not observe in any other case).

I was able to get the preparation out of its depression in the usual way. On the 27th of July it began once more to feed and to throw off

buds (Pl. XII, fig. 35). But now both heads took part in the catching of the foot—the individuated abdominal end of the old brown Hydra (*A*) and the newly formed head end (*B*). Formerly (Pl. XII, fig. 32) the new mouth used to pass over almost immediately into the bifurcating abdominal section. This time, however (Pl. XII, fig. 35), a rather large part of the new abdominal region had individuated between the new head and the bifurcation.

The common foot became much shortened in consequence. Not a trace was left of the red substance on the surface, either in the former stalk, or the hypostome, or the tentacles. The regulation of the number of tentacles was going on its own way.

The part that formed the junction between the two heads continued its de-differentiating process. It began once more to throw off buds towards the one head as well as towards the other. The more of active live substance it spent the fainter grew its colour, and began to remind one by its looks of the common inactive stalk.

Pl. XIII, fig. 36 (July 28th, the 30th day), represents the moment when the de-differentiating portions of the connective part had approached the body of the regulated Hydra (*B*) quite closely. The liberated, active, live substance had all been used up to form about eighteen to twenty buds. All these buds were typical *oligactis*, as there were no traces of the red substance to be found either in the connective parts, or in those of the body which were drawn towards the head *A*.

Of special interest were the changes which had occurred in the regulated Hydra. It had formed a large abdominal region and a regular stalk, typical of *oligactis*. We must not forget that in order to build up its head, it had used the hypostome of the red Hydra. And it must also be remembered that at a certain moment after the operation the red hypostome had called into existence the rudiments of the brown tentacles (Pl. XI, figs. 22, 23). These latter produced the brown portion of the hypostome, while this hypostome destroyed not only the red hypostome that had given it life, but in growing downwards, its brown substance brought down to nothing all the visible elements of the red Hydra. Thus the red Hydra had called up a "spirit" which it could not get rid of, being obliged to give way before it. The "incantations" which call up "spirits" run in strict forms—these are the form-determining stimuli which come from the edge of the hypostome, that organizing centre, and possess a strictly specific character. Their activity bears the same mechanical and automatic character as the various tropisms in the simplest organisms, or various reflexes in insects. These stimuli work faultlessly, but often quite aimlessly. And in the above-mentioned



instance, the capacity of the hypostome to raise the number of the tentacles to its usual figure, good in itself, became a source of peril to the organism that had made use of this power.

But what had not taken place in all the other heteroplastic grafting experiments, did not happen here either: the total disappearance of the elements of the red Hydra did not take place. The entire abdominal section of the red Hydra entered into the component parts of the body of the new Hydra (*B*). The foot of the red Hydra came up to the connective angle of the discoloured connective part (Pl. XIII, fig. 36) and this fact became the cause of the production of a new budding zone, which was immediately surrounded by five new buds. I remind the reader that in this part we have already seen three new buds growing up on the preparation—one normal *oligactis* bud and two sectorial chimaeras (Pls. IX and XI, figs. 2, 24, 27). The new buds, however, either bore within them, or united within themselves, the characters of both *vulgaris* and *oligactis* (*B*4, *B*6), or were true *oligactis* (*B*5), or bore an indefinite character (*B*7, *B*8).

I should like to mention one more stage (August 2nd, the 35th day)—Pl. XIII, fig. 37. The chimeric head (*B*) was finishing its work of regulating the number of tentacles. There were six of them in all; two of them had begun growing into one, and a new one had begun to sprout. The budding zone had already risen above the connecting channel; on it sat only one, the fifth of the newly produced buds with eight tentacles. The normal head (*A*) was in no way distinguished from a usual *oligactis*; in the budding zone which was approaching it, there were three buds, also typical *oligactis*. The whole preparation grew to enormous dimensions, about 3 cm. Both components were on the whole behaving quite independently of each other; they caught the food and contracted, each after its own fashion. But the canal in the discoloured connecting portion still united the abdominal parts of both components.

All further processes amounted to a mere regulation of the number of tentacles on the head of the chimaera, to the separation of a whole series of buds from the one component as well as from the other. (Over the budding process I shall have occasion to stop a little longer later on.) All further investigations were unfortunately interrupted by circumstances not dependent upon me. On August 12th the preparation was found in so low a condition that it was impossible to revivify it, and on August 14th, i.e. on the 47th day after the operation, the whole crumbled into pieces.

7. *Another Example of the Regulation of  
a Chimaera by Insertion.*

I have already pointed out that the pictures obtained from specimens regulated by means of insertion were so varied that it would take many pages to give individual descriptions of each. I shall therefore limit myself to the description of one more example where I discovered several strange features.

As the result of the insertion of a brown six-tentacled Hydra into a red one with seven tentacles (Exp. 178, June 30th, 1922) I obtained a very good compound of the two component parts (Pl. XIV, fig. 38), the tentacles of *vulgaris* surrounding the abdominal portion of the brown Hydra in a regular wreath.

The separation of the two components took place as follows: the inner, brown Hydra split in half and began to crawl out of the red one through two apertures (see Pl. XIV, fig. 39). But at the same time the red walls of the surrounding "muff" began to turn a dull colour, the impression being as if the matter of the brown Hydra were oozing through the walls of the red one. The crown of red tentacles underwent no change whatever. Without histological investigations, however, it is difficult to speak with precision of the inner condition of this phenomenon, all the more as I observed it only once in my heteroplastic operations. I have named this phenomenon the "association<sup>1</sup>."

This "association" acted evidently upon a comparatively small portion of the walls of the red Hydra. Those portions of the walls of the Hydra which remained untouched by this process crept down to one side.

The most extraordinary fate, however, undertook the head part. The tentacles kept their circular position, but the parts of the brown Hydra which were pushing out, tore the hypostome in two (Pl. XIV, fig. 40); the part of the hypostome which was carrying three tentacles and the remaining portion of the red wall of the body, passed on to one side of the abdominal region; while the other remained on the opposite side with four tentacles. Each portion began to form into a separate head. (I must here remind the reader, that each tentacle of a Hydra with its own hypostome portion, is capable of reproducing the whole Hydra.) During this process, the red head with four tentacles drew out of the adjoining wall of the brown body two brown tentacles.

<sup>1</sup> I made my insertion experiments for a definite purpose: I wished the inner component to be perfectly "associated" with—absorbed as it were, by—the walls of the outer body. Usually, the components separated. But in a few experiments with homoplastic operations (with the species *oligactis*) I succeeded in getting a perfect association.

The outer appearance of the regulation process changed unusually rapidly. On the 5th day after the operation (Pl. XIV, fig. 41) there was not the slightest trace left in the preparation, of the abdominal part of the red Hydra. The red pigment had been preserved only in the hypostomes of the newly fashioned heads in the tentacles, and in a pair of degenerating testes.

But even here the brown elements had already begun their attacking movement. The portion with the four tentacles had formed a regular head (Pl. XIV, figs. 41, 42), but half of the newly fashioned hypostome had already been seized by the brown substance. And more than this, one of the red tentacles had been already lifted up above the hypostome by the rudiment of a brown tentacle that had grown up within its base.

During the following two days the red substance became almost entirely destroyed even in the hypostomes of the newly formed heads. I have illustrated this in only one figure (Fig. 43), which is intended to show clearly the process of substitution of brown tentacles for red ones.

The remaining regulation processes took an exceedingly complicated course. The preparation had finally the following appearance: the greatly elongated Hydra bore in the middle of the abdominal portion, above the budding zone, two side heads, of which one very soon subdivided into two. On each of these three heads the tentacles formed figures of most extraordinary design. Lower down within the budding zone, buds were beginning to separate themselves from the parent body, as usual.

Everything I said when describing the last experiment, about organizing centres, form-determining stimuli, the interchange of tentacles, about the appearance of heteromorphoses—all would be equally applicable to this case. I must observe that during their substitution the red tentacles were either carried along on the tips of the brown ones, or were caught up by them after the brown ones were themselves sufficiently developed (Pl. XIV, fig. 44).

A short time after this, the budding zone of the preparation was lifted up to the level of the region where the association of the substances of the red Hydra had taken place; and here the budding activity was freed and immediately produced buds of two types—the pure *oligactis* and a compound of *oligactis* and *vulgaris*. But when the budding zone, in its upward movement, reached the three “regulation” heads, the investigation of the regulation details became almost impossible. The buds that were being thrown off in great numbers; the new heads that were unexpectedly separating themselves from various portions of the body;

the three older heads which were most fantastically regulating themselves into other shapes; all these had grown into one inextricable lump of expanding and contracting Hydras, which it was impossible to separate into its component elements.

But the original brown Hydra did not seem to notice at all these tumultuous proceedings which were taking place in the lower part of its abdominal region. It fed excellently, and grew in proportion.

And again there was noticed the phenomenon which we met in the preceding experiment—the abdominal part began to throw out buds above its regular budding zone, thus forming a new belt.

Evidently there exists a definite proportional relation between the hypostome and the size of the abdomen, analogous to that which exists, according to R. Hertwig, between the nucleus and the protoplasm.

This relation fixes the limits of the normal growth of the Hydra. As soon, however, as the balance of this relation inclines towards the abdominal part—as a result of good nutrition, assimilation and growth—the regulation mechanism asserts its rights, and removes the surplus living matter in the shape of buds.

In cases similar to the analyzed experiment—the budding zone was wanting or, strictly speaking, it was divided from the hypostome by a complicated mass of Hydras undergoing the regulation process. The abdominal region was therefore much increased in size. But as soon as its volume overstepped certain bounds, there began the de-activation of the body-wall substance—a new budding zone was produced, and a series of buds was thrown off from the centre of the abdominal part. As a consequence of this a comparatively small portion of the abdomen, under the fundamental hypostome, became separated into a new abdominal part, absolutely corresponding in size to that of the normal Hydra.

In the preparation in question, the victory was won at last by one of the head ends out of the complex mass of Hydras under regulation. This head became much elongated, and pushed aside or threw off the other component of the lumpy mass, and individualized itself into a large Hydra on the other side of the fundamental Hydra's abdominal region which was growing inactive.

I print two illustrations showing how in this newly created intermediate "stalky" zone a sole was formed (Pl. XIV, fig. 45), how glandular cells grew up in it; and how it became attached after this to the substratum (Pl. XIV, fig. 46). A few days after this juncture the separation of the two component parts was so much furthered that, while the preparation was

being removed from its vessel, it split into two separate Hydras in the foot region.

One of the components—the upper one—which corresponded to the original Hydra—presented no special points of interest, but the lower one was the outcome of most complicated regulating processes. It carried within itself the elements of the “associated” red Hydra; and, judging from its origin, it might be called a cytomictical chimaera; its chimeric character showed itself already during the budding processes. I was, however, unable to follow up its further transformations.

## II.

### 8. *The Budding of Cytomictical Chimaeras.*

In the foregoing account we saw that whatever the starting operation of heteroplastic grafting was—whether complantation, insertion or dissociation; and whatever course the regulating process might take—the result always showed the disappearance of the visible elements of the red component and the transformation of the original brown Hydra into a cytomictical chimaera. The process of regulation had an “equifinal” course, to use Driesch’s term. The difference in the statements arises from the fact that we put into our use of the term a very definite and realistic meaning.

The budding of such organisms presented a specially high interest, which arose from the following circumstances.

The account of Hydra budding, still found in many manuals, representing it as of primitive simplicity, i.e. how two layers of the wall substance of the Hydra are pushed out in the form of a little projection which grows into a bud, is giving way before the investigations of Hadzi (1910) and Tannreuther (1908–9) and is changing into the wider view of a more complex process.

In the opinion of these authors the chief part in the formation of new buds belongs to the *I*-cells (= interstitial cells). These cells form special germs of future buds. The budding process is but an activating into vitality of these germs. The principal mass of the bud in formation is obtained from the *I*-cells. They furnish both the ectoderm and entoderm with all their derivatives.

This phenomenon has been investigated anew lately in Korschelt’s laboratory. In his latest researches (published 1917) Korschelt devotes his attention to the question of asexual generation. Concerning the

budding process of Hydras, this author shares the view of Hadzi and Tannreuther.

The processes that have been investigated during the budding of cytomictical chimaera have confirmed this opinion.

The cytomictical chimaera differed nowise in its appearance from the common brown Hydra. It is usual to think that whenever some kind of chimaera is developed as a result of the grafting, or growing together, of two organisms, this chimaera must be considered as an individual of the first generation (= "a grafted hybrid") from two originating components.

If this supposition be in itself questionable, it is all the more so with regard to cytomictical chimaeras. For in these cases one of the organisms can be considered as having been dissolved as it were in the other, although the other remains evidently unchanged in its original constitution. That is why, when studying the process of generation, I have taken the cytomictical chimaera as the primary organism, and have named it "parental" (*PCh*).

With intense impatience did I wait for the beginning of vegetative generation in this regulated preparation; and when in the year 1921 I was able to establish the fact of the appearance of unusual buds upon it which showed signs of mixed features of the red and the brown Hydra, I hoped to have found an example of the heredity of acquired qualities, viz. that the brown Hydra through having "associated" with the red one, had taken over from it its manner of budding and had begun to transmit it to its own posterity.

But experiments of a wider range which I made in 1922, proved that the source of the investigated processes was on the one hand much simpler than I had assumed, and on the other much more complicated.

I must refer once more, briefly, to the different kinds of budding.

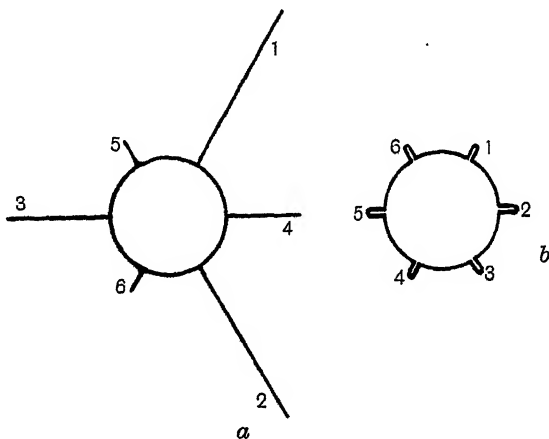
The species *oligactis* puts forth, as is well known, first two tentacles with its bud, then appear in succession the 3rd and the 4th, after which the 5th and the 6th appear more or less simultaneously. This order and their setting are best illustrated in the appended scheme (Text-fig. 6 a). I note the order of the appearance of the tentacles thus:  $1=2>3\geq 4>5=6$ . The bud becomes detached when bearing four, seldom five or six long winding tentacles. Those tentacles which fall short of the number make their shortcoming good very quickly<sup>1</sup>.

The species *vulgaris* produces all its tentacles at once (see the scheme),

<sup>1</sup> Among hundreds of specimens that have passed through my hands I have found only one bud of *oligactis* with seven tentacles.

a fact which I denote thus:  $1 = 2 = 3 = 4 = 5 = 6 = 7 = \dots$ . The number of tentacles is five, six and not infrequently seven, eight or even nine. These tentacles are straighter and thicker than those of *oligactis*; their number may reach ten to twelve in the course of their lifetime.

The position of the buds is also rather characteristic. In the species *oligactis* the buds come out in a special budding zone, along the border-line between the stalk and the abdominal region, and climb up one after the other in a rather close spiral. The species *vulgaris* has no stalk and consequently no definite budding zone either; the buds grow up in the



Text-fig. 6. The mode of appearance of tentacles on the buds of *oligactis* (a)  $1 = 2 > 3 \geq 4 > 5 = 6$  and *vulgaris* (b). Maternal Hydra is to the right.

lower portion of the body, and the position of the buds is not spiral, but opposite to each other, verticillate or indefinite.

Passing on to the description of the phenomenon of propagation and vegetative segregation of cytomitical chimaeras, I deem it necessary to explain first the various terms used by me.

I call the first generation not  $F_1$  but  $G_1$  as I consider the first denomination to be correct only in the case of sexual generation, where there are really parents (*Parentale*) and children (*Filiale*). But in the case of the sexless generation, as in the instance described by me, where we have a *pure line* from a single parental organism (*PC<sub>h</sub>*), it seems best to mark the generations as they succeed each other, by a more neutral term, viz.—generation— $G_1, G_2, G_3$ , etc.

Buds are marked by me by figures, as they appear on the parent organism:  $B_1, B_2, B_3, \dots B_4$ . The number of figures in the formula shows the number of generations, thus: one figure ( $B_1$ ) =  $G_1$ ; two figures

( $B1.1$ ) =  $G_2$ ; six figures ( $B1.1.1.1.1.1$ ) =  $G_6$ , and so forth. The last figure in the formula determines the order of the bud in the given generation. Thus the formula  $B2.2.1.6.7.1.6$  marks the sixth bud in the seventh generation of the line  $B2$ . The figures which come before the last 6 show the course along which the last, or seventh, generation was brought forth.

In the following account I shall analyze in detail the genetics of the cytomitictal chimaera which I obtained as the result of my insertion experiment described first (Exp. 164). About the propagation of other chimaeras I must content myself to say but a few brief words.

### 9. The First Generation. $G_1$ .

(Genealogical Table I.)

A week after the operation we noticed a swelling of the ectoderm (Pl. XV, fig. 47) in the lower part of the abdomen of the regulated chimerical preparation, which generally precedes the appearance of a bud ( $B1$ ). On the following day this swelling grew into a salient projection bearing two tentacles; which proved that the activated germ was that of the *oligactis* bud. Above this bud there was formed a second ( $B2$ ) about the nature of which nothing could be said.

On the following day the nature of the first bud became still better defined, when perpendicularly to the level of the first two tentacles, there arose two small beginnings of new tentacles. The second bud ( $B2$ ) drew our attention at once by its unusual manner of producing tentacles. Around the future hypostome there appeared simultaneously six small germinating projections of the future tentacles (Pl. XV, fig. 51).

This manner of breeding is characteristic, as we know, of the species *vulgaris*, just as the manner mentioned previously is characteristic of the species *oligactis*. I immediately conceived the possibility of an original vegetative segregation of the chimerical preparation, the cause of which was at that time still obscure to me. This made me follow the budding process with heightened interest.

The first bud was thrown off like the typical stalk-bearing hydra with four long wriggling tentacles.

The second bud ( $B2$ , Pl. XV, fig. 48) grew to a considerable size, but all the six tentacles had, as before, the same length, being all rather short and straight, and sitting round like a cone, with their ends turned towards the aperture of the mouth.

The third bud ( $B3$ , Pl. XV, fig. 48) was formed in the ordinary way, typical for *oligactis*.



The fourth (*B 4*) again showed peculiarities. One of the tentacles was distinguished by its greater length, and appeared before the next three, which all grew up at the same time.

There is nothing to be said about the nature of the fifth bud (*B 5*).

The second bud (*B 2*) grew a stalk three days after its birth and broke from the maternal organism. A merely superficial examination brought to light the characteristic position of the tentacles and their strange manner of holding themselves. They had pulled themselves out slightly, but were bent in straight lines to the sides and a little upwards, still forming a pretty regular cone. But the lower part of the body—the stalk, the sole, the clearly defined border between the stalk and the abdominal region forming the budding zone—all these were typical marks of species *oligactis*. Indeed, the whole outward appearance of the individual pointed to the species *oligactis*.

Such Hydras, which unite within themselves the complex characters of the *oligactis* species (general aspect, stalk and budding zone) with those of the species *vulgaris* (the manner of sprouting of the tentacles, their number and position, partly also the position of the buds) I have named “oligactoids,” from an analogy with the speltoids and speltoid-chimaeras of Nilsson-Ehle and Åkerman (1920).

Thus during the budding period the maternal chimaera (*PCh*) suffered an odd kind of segregation—into pure *oligactis*, which I shall mark in future by the symbol *P* (= *Pelmatohydra oligactis*), and into oligactoids *PH* (= *Pelmatohydra* + *Hydra vulgaris*). It is necessary to remember that in nature oligactoids are never met with. While passing a living substance through a chimerical preparation I succeeded in synthesizing a special, absolutely new, “species” of the Hydra.

The physical basis of the origin of these oligactoids is perfectly clear. Once the parental organism represented a cytomictical chimaera (*PCh*), and there were simultaneously present *I*-cells of both species within the budding zone, it becomes absolutely comprehensible that when these mixed budding germs become active, the result must certainly have contained features of both species.

The segregation of the parental organism might very rightly be termed vegetative. Plant chimaeras also undergo vegetative segregation, but there is one characteristic feature which assigns to my chimaeras a special place. Plant chimaeras always bring forth both components in a pure state; but in my chimaeras one of the components burst off in a pure state, while the other was replaced by strange organisms of a mixed nature, which retained also in the future the intermediate, mixed

character of the original organism which brought them very near to the true graft-hybrids.

Pl. XV, fig. 49, represents one of the moments of active vegetative segregation. *B 3 = oligactis*, *B 4 = oligactoid* (the real nature of this bud was revealed later); *B 5 =* is a typical oligactoid with the commencement of eight tentacles; *B 6 = oligactis* with the beginnings of two tentacles; *B 7* and *B 8 =* bud projections of an indefinite kind. I should like to draw attention to the irregular position of the buds. Instead of a regular upward climb of the buds over the abdominal region along a spiral line, we see that *B 8* had appeared lower than *B 5* and opposite to *B 2*. The upper group of buds had arranged itself in a pretty verticil. The red component *H* (= *Hydra vulgaris*) had not yet freed itself from the chimerical preparation, although it had begun budding of its own accord (*B'1*).

During the following days the preparation grew rapidly, budding plentifully all the time. No order of any kind could be discerned in the putting forth or throwing off of buds *P* or *PH*—*B 7* resulted in *P*; *B 8*—*PH* (Pl. XV, fig. 50); then followed *PH*, *P*, *PH*, *P*, *P*, *P*, *PH*. The sequence of buds is best illustrated by the appended scheme and by the records of investigations to which I beg to refer the reader.

On the 17th of July the preparation had reached its maximum growth (Pl. VII, fig. 52). When at rest the preparation measured about two centimetres. At that time it was bearing upon it *B 16*—*P*, *B 17*—*PH\**, *B 18*—*PH*, *B 19*—*P*, *B 20*—an undefinable commencement of a bud. I draw the reader's attention to *B 17*, a bud marked *PH\**. Of its five tentacles one was very striking for its length (Pl. XV, fig. 52 *a*). The characteristic difference revealed itself during the very first moments of production. I have marked this variety of oligactoids by a small asterisk, they are *oligactoids with a particularly long tentacle*.

Meanwhile the red component (*H*) had slid down to the inactive stalk of the chimerical preparation. As may be seen in the illustration (Pl. XV, fig. 52), it produced an egg which became fertilized. This process had no influence whatever on the generative powers of the original preparation.

The next few days the preparation underwent a slight depression—it threw off a series of buds, freed itself from its red component and decreased considerably in volume.

On July 23rd the side of the animal portion proved slit open by *Daphnias* with which the stomach was shown to be filled. The big slit was rather quickly drawn together by the animal's tissues, but in consequence of the uneven growth, the head end proved to have got near the budding zone; and in the opposite wall of the body there had grown up a blind

sac (Pl. XV, fig. 53). (One of the tentacles had wandered down from the hypostome to a side wall.)

This circumstance had evidently influenced the bud germs present in the walls of this bag so that they became active. (The projecting part of the abdominal region could most easily have become regulated by the simultaneous casting off of live matter in the shape of buds.) Pl. XV, fig. 54, shows how in this zone four buds sprang up at the same time—*B* 30 to *B* 33.

Part of these buds were thrown off in the normal way, but some of them underwent a stranger fate. Thus, for instance, the budding zone slipped past the base of bud *B* 26, and settled consequently on the stalk (Pl. XV, fig. 55). *B* 33 remained on the blind excrescence of the abdominal region, which had become drawn out into a thin shoot: this was a very strange kind of pedestal for a bud.

During the following days plentiful budding lifted the zone above the excrescence which united *B* 33 with its parent organism (Pl. XV, fig. 56). It is characteristic that this excrescence itself developed a bud (*B* 49), as if for the purpose of hastening on the regulating processes.

The buds thrown off during this budding period belonged chiefly to the types of *PH* and *PH\**; of pure *P*'s there were comparatively few. On August 7th there were fixed to the preparation (Pl. XV, fig. 56) *B* 26 of an unknown type, *B* 33—type ?, *B* 75—*P* (with six tentacles), *B* 46—*PH* (with six tentacles), *B* 47—*PH* (with seven tentacles), *B* 48—*PH* (six tentacles), *B* 50 and *B* 51 of undefined nature.

Circumstances over which I had no control brought a week's interruption into my investigations. Meanwhile the preparation had cast off all its buds with the exception of two (*B* 26 and *B* 49) which had crawled down to the inactive stalk. Pl. XV, fig. 57, is freehand drawing, and does not represent the exact proportions of the preparation.

In connection with the characteristic features of this period of the preparation's life, there arose several considerations caused by the "colonizing tendencies" of the Hydra. It is usual to term "temporary colony" every Hydra which buds plentifully, and where the separate buds are for a time joined to the abdominal cavity of the maternal organism. It seems to me, however, that it would be correct to term the Hydra a "colony" only from the moment that the buds remain with the maternal organism in a lasting connexion. Such a lasting union is secured by the buds wandering across from the budding zone to an inactive stalk. Therefore the most characteristic feature for a "colony" is the effectiveness of the union of separate zooids by means of a branched inactive stolon.

Pl. XV, fig. 57, represents just such a true colony: a forked stolon unites three separate, independent zooids. This drawing helps to form an accurate idea of the origin of "colonization" in general.

Plentiful feeding brought the preparation back to its previous condition. Budding recommenced, accompanied by segregation (Pl. XV, fig. 58, August 23rd): *B* 60—*PH*, *B* 61—5 (with five tentacles). The buds *B* 26 and *B* 49 moved down to the very base of the stalk, become individuated, but still did not split off.

After this the preparation was attacked by the infusorian *Kerona*. Having parted with some of its buds, it became greatly depressed, during which state it lost its stalk and two buds which were sitting at its base—*B* 26 and *B* 49.

I succeeded in washing off the *Keronas*, the preparation recovered, made a new stalk, and turned into a small normal *Hydra* with six tentacles. On the 4th of September I finished with it; it was the 68th day after the operation.

During the whole period of its long life the original animal which I had used for my experiment, having changed into a cytomictical chimaera developed 65 buds (Table I):

16 buds of the *P* type,  
 37 " " *PH* "  
 7 " " *PH\** "  
 5 " of indefinite type<sup>1</sup>.

Summarizing the acts of segregation, we find the following relations:

$$44 PH:16 P = 2.75 PH:1 P.$$

It is remarkable that during the segregation, the component *H* did not once separate in a pure condition.

TABLE I.

Type of buds	Number of tentacles								Number of buds
	?	3	4	5	6	7	8	9	
<i>P</i>	—	—	9	5	2	—	—	—	16
<i>PH*</i>	—	—	1	5	1	—	—	—	7
<i>PH</i>	(1)	—	—	8	19	8	1	—	37
?	(1)	—	1	2	1	—	—	—	5
<i>G</i> <sub>1</sub>	2	—	11	20	23	8	1	—	65

<sup>1</sup> In my tables these *Hydras* are marked with a note of interrogation. I did not succeed in ascertaining their nature.

This was owing to the absence of investigations of the nature of rudiments of tentacles. The budding phenomenon might no doubt give a definite answer; but these *Hydras* died before this process began.

It might also have been because the *Hydras* were still in a state of indefinite budgerms when the parental organism that bore them perished.

I postpone any discussion of these proportional relations to the end of my description of the process of vegetative generation.

#### 10. *The Second Generation. $G_2$ .*

In the beginning of my researches I followed closely the fate of each representative of the first generation. But it soon became clear that whenever an *oligactis* ( $P$ ) was thrown off, it always bred true. Their rate of propagation was even considerably quicker than the budding of oligactoids. The second generation of the  $P$  type immediately gave birth to the third; the latter to the fourth, and so on. I followed the course of development of some individuals down to the  $G_6$ ,  $G_7$ , and all through they kept their genotypical purity, giving all the time only  $P$  buds.

Therefore, as the number of specimens under cultivation increased, I decreased the number of generations under control to two; towards the end I was often satisfied with watching the development of the earliest stage of tentacle life only; and frequently, by simply noticing the look of the budding excrescence, I was able to predict the way its development would follow.

My chief attention, however, was directed towards the fate of the oligactoids, the commencement of whose budding I waited for with heightened interest.

*Line B 2, type PH, 9. vii.* (Genealogical Table II). On the first oligactoid of line  $B 2$  I noticed on the very next day after its separation the beginnings of two buds ( $B 2.1$  and  $B 2.2$ ). On the upper end of the first bud there grew up at once seven tentacles, on the second bud five. The third bud bore also immediately five tentacles, and the fourth seven. All these buds (type  $PH$ ) belonged to the second generation ( $G_2$ ) and all of them grew up out of the normal budding zone. The spiral position of the buds altered with the opposite position.

All the buds were thrown off the maternal organism two or three days after germinating. All of them formed, in a day or two, clearly marked stalks under the abdominal part. The character of the tentacles was the same as those on the parent oligactoid. This proved that the oligactoid of  $G_1$  had transmitted to its descendants in  $G_2$  the features it had received from its parent ( $PCh$ ), in their entirety.

The fifth bud bore only four tentacles. Their position was characteristic of  $P$  (Pl. XVI, fig. 59); only their dimensions differed somewhat (index  $1 > 2 > 3 > 4$ , and not  $1 = 2 > 3 \geq 4$ ).

I was obliged to admit that the oligactoids of the first generation had undergone a vegetative segregation. The capacity to emit buds of the

*P* type pointed to the presence of corresponding *I*-cells. This circumstance on the other hand proved that the "new species" obtained during the grafting experiments, i.e. the oligactoids, were in fact the same kind of cytomictical chimaeras, as the first parent organism. The *I*-cells obtained from this stock had kept their genotypical purity even under the outer covering of an oligactoid.

We may say, therefore, that the oligactoids represent as it were true, grafted, and at the same time intermediate "hybrids"; their segregation in the second generation points to their heterozygous character. We must keep in mind, that at the bottom of the "hybridity" and of the "state of heterozygosis" there lies a peculiar chimerical organisation (a cytomixis).

The fifth bud (*B* 2.5) was thrown off with its four original tentacles, which had by that time all assumed the same dimensions.

The hypostome of the original oligactoid (*B* 2) had gone through an interesting change. One of its tentacles had grown very much, like those of type *PH*\*; after which there appeared a new tentacle, the seventh.

The further state of the budding process is illustrated in the Table of Records. Most interesting was the fate of bud *B* 2.8. It grew up quite normally within the budding zone, with six tentacles (*PH*). But afterwards, before its final individuation it must evidently have fallen into an accidental slit in the wall of the maternal organism. The wound was rapidly drawn together by the neighbouring tissues; but these same tissues covered the bud *B* 8, that had got into the opening, as well. Thus there took place before my own eyes, under circumstances that had arisen in Nature herself, a natural growing together—complantation—(Pl. XVI, fig. 60) between the parent organism and its bud. The hypostome of the bud was placed a little below the hypostome of the fundamental Hydra; the foot was sticking out still lower from the wall of the Hydra; the abdominal portion of the bud was shining through the abdomen of the outer fundamental Hydra.

Later on the abdominal part of the bud which had been seized, was absorbed by the parental tissues. The hypostome became individualized as a second head part under the original head (a new manner of bringing about "two-headed" Hydras). The stalk had lost all connexion with its head, and had gone over to the other side (Pl. XVI, fig. 61). Into the adjoining tissues of the abdominal portion of the brown Hydra the stalk had installed a special budding zone (a new "organizing centre") in which buds began to individuate (*B* 11 = *P*); I am speaking of a *new* budding zone, as the original zone had not yet risen to that height.

TABLE II.  
*G<sub>1</sub> from PCh 164. Table of Records*

<i>G<sub>1</sub></i>	Date of appearance	Date of casting off	Number of tentacles, Type:			Beginning of budding ( <i>G<sub>2</sub></i> )	Number of buds of <i>G<sub>3</sub></i>			Perished	Remarks
			<i>P</i>	<i>PH</i>	?		<i>P</i>	<i>PH</i>	?		
<i>B</i> 1	5. vii.	8. vii.	4	—	—	—	—	—	—	—	True <i>P</i> . From fundamental <i>obligatis</i> . = Line <i>B</i> 2. On 7th simultaneous appearance of 6 tentacles. = Line <i>B</i> 4; 3 tentacles simultaneous, a particularly long one. Beginning of budding process with retardation. Appears above the budding zone. Below <i>B</i> 5.
2	6. vii.	9. vii.	—	6	—	10. vii.	1	10	1	6. viii.	
3	8. vii.	10. vii.	5	—	—	—	—	—	—	—	
4	8. vii.	10. vii.	—	4*	—	16. vii.	2	5	1	23. vii.	
5	8. vii.	10. vii.	—	8	—	—	—	—	—	20. vii.	Below <i>B</i> 4— <i>B</i> 7 (1). Above all preceding buds. Simultaneously 5 tentacles. On the level of <i>B</i> 6— <i>B</i> 7. Below <i>B</i> 13.
6	9. vii.	11. vii.	4	—	—	—	—	—	—	—	
7	9. vii.	12. vii.	4	—	—	16. vii.	—	—	—	27. vii.	
8	9. vii.	12. vii.	—	5	—	—	—	1	—	20. vii.	
9	10. vii.	13. vii.	4	—	—	—	—	—	—	—	Not so well marked type <i>PH</i> . First appearance with 4 small and irregular tentacles; cast off with 6 tentacles. } Opposite position. Remained 9 days on the stalk. Remained one month on the stalk. Appears with 4 tentacles, irregular disposition. 29. vii. a forked tentacle. 19. viii. added 1 tentacle. Degenerated during depression.
10	10. vii.	13. vii.	4	7	—	21. vii.	—	—	—	24. vii.	
11	11. vii.	15. vii.	4	—	—	—	—	—	—	—	
12	12. vii.	16. vii.	5	—	—	—	—	—	—	—	
13	13. vii.	16. vii.	4	—	—	—	—	—	—	23. vii.	Line <i>B</i> 17. One tentacle particularly long. With one forked tentacle. First appearance with 4 small and irregular tentacles; cast off with 6 tentacles. } Opposite position. Remained 9 days on the stalk. Remained one month on the stalk. Appears with 4 tentacles, irregular disposition. 29. vii. a forked tentacle. 19. viii. added 1 tentacle. Degenerated during depression.
14	13. vii.	17. vii.	4	6	—	20. vii.	—	2	—	—	
15	14. vii.	17. vii.	5	—	—	—	—	—	—	4. ix.	
16	15. vii.	18-19. vii.	—	5*	—	19. vii.	12	25	4	27. viii.	
17	16. vii.	18-19. vii.	—	7	—	—	—	—	—	—	Not so well marked type <i>PH</i> . First appearance with 4 small and irregular tentacles; cast off with 6 tentacles. } Opposite position. Remained 9 days on the stalk. Remained one month on the stalk. Appears with 4 tentacles, irregular disposition. 29. vii. a forked tentacle. 19. viii. added 1 tentacle. Degenerated during depression.
18	16. vii.	24. vii.	5	—	—	—	—	—	—	—	
19	17. vii.	18-19. vii.	—	5*	—	22. vii.	5	6	4	24. viii.	
20	17. vii.	21. vii.	—	5*	—	22. vii.	10	2	11	24. viii.	
21	18-19. vii.	21. vii.	—	6	—	—	—	—	—	27. vii.	Gave 2 buds of type <i>P</i> . } Appearance of tentacles not examined.
22	18-19. vii.	21. vii.	—	—	—	—	—	—	—	—	
23	21. vii.	23. vii.	—	6	—	—	—	—	—	4. viii.	
24	21. vii.	25. vii.	4	—	—	—	—	—	—	28. vii.	
25	22. vii.	1. viiii.	—	5	—	—	—	—	—	4. viii.	Gave 2 buds of type <i>P</i> . } Appearance of tentacles not examined.
26	23. vii.	24-27. viii.	—	—	4	—	—	—	—	—	
27	25-26. vii.	28. vii.	—	5*	—	—	—	—	—	31. vii.	
28	27. vii.	29. vii.	4	—	—	—	—	—	—	—	
29	28. vii.	30. vii.	4	—	—	—	—	—	—	31. vii.	Gave 2 buds of type <i>P</i> . } Appearance of tentacles not examined.
30	29. vii.	1-2. viii.	—	7	—	—	—	—	—	8-23. viii.	
31	29. vii.	1-2. viii.	—	6	—	—	—	—	—	8-23. viii.	
32	29. vii.	1-2. viii.	—	7	—	—	—	—	—	5. viii.	

TABLE II (continued)

$G_1$ B 33	Date of appearance	Date of casting off	Number of tentacles. Type:			Begin- ning of budding ( $G_2$ )	Number of buds of $G_3$			Perished	Remarks
			$\overline{P}$	$\overline{PH}$	?		$\overline{P}$	$\overline{PH}$	?		
	29. vii.	8-11. viii.	—	—	5	—	—	—	—	12. vii.	Remained long on a prolongation of the abdominal part of parental chimaera.
34	30. vii.	2. viii.	—	6	—	3. viii.	1	1	—	7. viii.	Buds collected on 24. viii.
35	31. vii.	2. viii.	—	5*	—	?	1	6	—	24. viii.	
36	31. vii.	2. viii.	—	6	—	—	—	—	—	3. viii.	{ Vertical consisting of three PH. Added to Cult. XV. 5 buds of type P. Added to Cult. XV. 1 bud of type P.
37	1. viii.	2. viii.	—	7	—	—	—	—	—	5. viii.	
38	1. viii.	2. viii.	—	6	—	—	—	—	—	8-23. viii.	
39	1. viii.	3. viii.	—	5	—	—	—	—	—	3. viii.	
40	1. viii.	3. viii.	—	5	—	—	—	—	—	3. viii.	On base of prolongation bearing B 33. Disappeared during depression 24-27. viii.
41	2. viii.	4-5. viii.	—	6	—	?	—	3	—	24. viii.	
42	3. viii.	4. viii.	6	6	—	5. viii.	(5)	—	—	6. viii.	
43	4. viii.	6. viii.	—	6	—	—	—	—	—	—	
44	4. viii.	6. viii.	5	—	—	6. viii.	(1)	—	—	6. viii.	Added to cult. of oligactoids (XXIII).
45	5. viii.	7. viii.	6	—	—	—	—	—	—	12. viii.	
46	5. viii.	7. viii.	—	6	—	—	—	—	—	12. viii.	
47	5. viii.	7. viii.	—	7	—	—	—	—	—	12. viii.	
48	5. viii.	8-11. viii.	—	6	—	—	—	—	—	12 viii.	Dissolved during depression. Added to Cult. of oligactoids (XXV). Dissolved during depression.
49	5. viii.	24-27. viii.	—	6	—	—	—	—	—	—	
50	6. viii.	8-11. viii.	—	—	6	—	—	—	—	12. viii.	
51	6. viii.	8-11. viii.	—	—	5	—	—	—	—	12. viii.	
52	14. viii.	18. viii.	—	6	—	—	—	—	—	24. viii.	Dissolved during depression.
53	16. viii.	19. viii.	—	6	—	—	—	—	—	28. viii.	
54	17. viii.	20. viii.	—	6*	—	28. viii.	—	1	—	2. ix.	
55	19. viii.	21. viii.	—	5	—	—	—	—	—	2. ix.	
56	19. viii.	21. viii.	—	7	—	—	—	—	—	2. ix.	{ Dissolved during depression. Added to Cult. of oligactoids (XXV). Dissolved during depression.
57	20. viii.	21. viii.	—	5	—	—	—	—	—	2. ix.	
58	20. viii.	22. viii.	—	7	—	—	—	—	—	23. viii.	
59	20. viii.	23. viii.	—	6	—	—	—	—	—	23. viii.	
60	21. viii.	24-27. viii.	—	6	—	—	—	—	—	24-27. viii.	4. ix. Five of the Parental Chimaera.
61	21. viii.	24-27. viii.	—	6	—	—	—	—	—	24-27. viii.	
62	24. viii.	27. viii.	5	—	—	—	—	—	—	28. viii.	
63	24. viii.	27. viii.	—	6	—	—	—	—	—	31. viii.	
64	27. viii.	27. viii.	—	?	—	—	—	—	—	31. viii.	Dissolved during depression.
65	28. viii.	—	—	—	—	—	—	—	—	31. viii.	
$G_1$	—	—	16	44	5	$G_2$	32	63	21	—	



It is interesting to note that later on the stalk of *B* 8 took upon itself the leading part in serving to attach the whole preparation (Pl. XVI, fig. 62).

On August 6th depression put an end to our investigations.

The sum total of buds produced in the second generation along the line *B* 2 was 12:

$$2 P : 9 PH : 1 ?$$

which proportion had to be altered later on into:

$$1 P : 10 PH : 1 ?.$$

*Line B* 4, type *PH\**, 10. vii. 22. (Genealogical Table IV). The bud *B* 4 out of *G*<sub>1</sub> was expelled from the maternal chimaera in the shape of a small starveling with four tentacles of unequal length. It was impossible to say what type it belonged to. After a week of careful tending, it had grown a little, and developed at first two, then a third tentacle, and began to bud.

During its short lifetime it added the following organisms to the second generation:

$$2 P : 5 PH : 1 ?.$$

Of its five *PH* four possessed one extra long tentacle each (type *PH\**).

*Line B* 17, type *PH\** (July 19th, Genealogical Table IV). This line kept throwing off buds of the second generation for nearly six weeks. Segregation was going on all the time; buds of the *P* type alternated more or less regularly with those of *PH*. I print Pl. XVI, fig. 63, as an example of a very active moment of vegetative segregation. During that time the oligactoid was covered with the following buds:

B 17.31—5 tentacles ( <i>PH</i> );
33—6        „        ( <i>PH*</i> );
34—6        „        ( <i>PH</i> );
35—4        „        ( <i>P</i> );
36—undefined.

Throughout the whole time there were brought forth:

$$12 P : 25 PH : 4 ?.$$

*Line B* 20, type *PH\** (July 21st), reminded me of the foregoing; only the number of *P* had increased:

$$5 P : 6 PH : 4 ?.$$

*Line B* 21 (July 21st). I was unable to ascertain the point of departure of this line at once after its separation, as its proportion of tentacles

( $1 > 2 \geq 3 = 4 = 5$ ) varied a little from the habitual scheme of the *oligactis* species. Two tentacles, especially, distinguished themselves from the rest by their unusual length. Therefore I marked it  $PH^{**}$ . The presence of two long tentacles indicated the prevalence of the  $P$  elements in the body of this oligactoid. The budding process confirmed my supposition. It resulted in:

$$10 P : 2 PH : 11?$$

Looking at all these numerical relations we notice that there was no special regularity in the appearance of the various kinds of buds. In some of the lines there was a strong preponderance of  $PH$ ; sometimes they were equalled in number by  $P$ ; and sometimes the predominance was on the side of  $P$ . Evidently everything depended upon the number of  $I$ -cells which had gone to form the oligactoid.

Adding all the individuals of  $G_2$ , those mentioned above, as well as those not mentioned here, but included in the list of Records, we obtain the following sum:

$$G_2 - 32 P : 63 PH \text{ (of which 13 } PH^*) : 21? \text{ or } 1 : 1.94.$$

In the second generation the index  $P : PH$  proved a little higher than in the first generation.

All this proves still better the fortuitous nature of numbers in vegetative segregation.

TABLE III.

Type of buds	Number of tentacles								Number of buds
	?	3	4	5	6	7	8	9	
$P$	1	—	8	17	6	—	—	—	32
$PH^*$	—	—	1	5	4	3	—	—	13
$PH$	1	—	—	9	20	16	4	—	50
?	2	1	2	5	10	1	—	—	21
$G_2$	4	1	11	36	40	20	4	—	116

### 11. The Third Generation. $G_3$ .

The oligactoids of the first generation had transmitted their outward form to the second ( $G_2$ ) without any change, transmitting at the same time also their heterozygous condition, i.e. the capacity of segregation into separate components—into true *oligactis* and into oligactoids. The numerical relation in  $G_1$  and  $G_2$  already showed a predominance of  $PH$ , which pointed to their “dominant” character. Nevertheless these “dominant heterozygous” forms ( $PH$ ) were less capable of living than their “recessive homozygous” forms ( $P$ ). While frequently the freed

TABLE IV.  
*G*<sub>2</sub> from *PC*h 164.

<i>G</i> <sub>2</sub>	Date of appear- ance	Date of casting off	Number of tentacles, Type:			Begin- ning of budding ( <i>G</i> <sub>3</sub> )	Number of buds of <i>G</i> <sub>3</sub>			Remarks
			<i>P</i>	<i>PH</i>	?		<i>P</i>	<i>PH</i>	?	
<i>B</i> 2. 1	10. vii.	12. vii.	—	7	—	—	—	—	—	Individualised as <i>PH</i> . Later, 1 tentacle grew longer. } Died in depression. Appearance of tentacles: 1 > 2 > 3 > 4; individuated: 1 = 2 > 3 = 4. Marked as <i>P</i> , segregation showed that it belonged to <i>PH</i> *.
2	10. vii.	13. vii.	—	5	—	16. vii.	4	17	—	
3	11. vii.	14. vii.	—	5	—	—	—	—	—	
4	11. vii.	15. vii.	—	7	—	—	—	—	—	
5	14. vii.	17. vii.	—	4*	—	18. vii.	11	8	5	
6	15. vii.	19. vii.	—	6	—	—	—	—	—	Attached with the side of its stalk. Remained longer on maternal Hydra. Natural coplantation with maternal Hydra. Individuated as head, and perished during the depression with fundamental Hydra.
7	16. vii.	24. vii.	—	7	—	—	—	—	—	
8	18. vii.	—	—	6	—	—	—	—	—	
9	21. vii.	1-2. viii.	—	6	—	—	—	—	—	From budding zone, organized by the stalk of bud <i>B</i> 8. Its 3 buds ( <i>G</i> <sub>3</sub> )—all <i>P</i> .
10	23. vii.	28. vii.	—	—	6	—	—	—	—	
11	25. vii.	28. vii.	5	—	—	6. viii.	—	—	—	
12	26. vii.	28. vii.	—	6	—	—	—	—	—	At first appearance of tentacles marked as type <i>PH</i> : 21. vii. seemed to be <i>PH</i> *. Budding process gave 9 buds ( <i>G</i> <sub>3</sub> )—all <i>P</i> ; in following generation ( <i>G</i> <sub>4</sub> )—all <i>P</i> .
Line <i>B</i> 2	—	—	1	10	1	<i>G</i> <sub>3</sub>	15	25	5	
<i>B</i> 4. 1	16. vii.	18. vii.	—	6*	—	19. vii.	4	25	7	
2	17. vii.	19. vii.	—	5*	—	20. vii.	2	9	3	
3	18. vii.	21. vii.	—	—	5	—	—	—	—	
4	18. vii.	21. vii.	5	—	—	—	—	—	—	
5	18. vii.	21. vii.	—	7	—	—	—	—	—	
6	19. vii.	22. vii.	5	—	—	23. vii.	—	—	—	
7	19. vii.	22. vii.	—	5*	—	—	—	—	—	
8	20. vii.	22. vii.	—	5	—	—	—	—	—	
Line <i>B</i> 4	—	—	2	5	1	<i>G</i> <sub>3</sub>	6	34	10	

TABLE IV (continued).

$G_3$	Date of appearance	Date of casting off	Number of tentacles.		Begin-ning of budding ( $G_3$ )	Number of buds of $G_3$			Perished	Remarks
			$P$	$PH$		$P$	$PH$	$P$		
$B 8.1$	16. vii.	20. vii.	—	6	—	—	—	—	27. vii.	
Line $B 8$	—	—	—	1	—	—	—	—	—	Perished with its bud.
$B 11.1$	21. vii.	—	—	6	—	—	—	—	27. vii.	
Line $B 11$	—	—	—	1	—	—	—	—	—	
$B 15.1$	20. vii.	22. vii.	—	7	—	—	—	—	23. vii.	
2	20. vii.	22. vii.	—	6	—	—	—	—	22. vii.	{ Two buds in opposite position.
Line $B 15$	—	—	—	2	—	—	—	—	—	
$B 17.1$	19. vii.	21. vii.	—	5*	—	—	—	—	8. viii.	
2	20. vii.	22. vii.	4	—	—	—	7	—	4. viii.	In 8 days gave 12 buds of type $P$ . In 5 days number of tentacles increased to 6.
3	20. vii.	23. vii.	—	7	—	—	—	—	31. vii.	Appeared with 6 tentacles; individuated with 7.
4	20. vii.	24. vii.	—	6	—	—	—	—	24. vii.	21. vii.—5 tent., 22—one tent. grew longer; 23—6 equal tent.
5	21. vii.	24. vii.	—	5*	—	—	6	1	2	Only one bud (in $G_3$ ) of type $PH^*$ .
6	21. vii.	24. vii.	—	7	—	—	—	1	—	Gave 2 buds ( $P$ ).
7	21. vii.	24. vii.	—	5	—	—	—	—	4. viii.	Tentacles: 1=2>3=4=5=6.
8	22. vii.	26. vii.	—	6*	—	—	—	5	5	Gave 5 buds of type $P$ ( $G_3$ ); following generation ( $G_4$ )— $P$ .
9	23. vii.	26. vii.	—	5	—	—	—	—	4. viii.	$G_3$ —4 $P$ ; $G_4$ — $P$ .
10	24. vii.	27. vii.	—	6	—	—	—	2	—	26. vii.—4 + 1 rud. tent., 27—5 + 1 rud. tent. $G_3$ —3 $P$ ; $G_4$ — $P$ .
11	24. vii.	27. vii.	—	5	—	—	—	—	4. viii.	
12	25. vii.	28. vii.	—	4	—	—	—	—	4. viii.	
13	26. vii.	29-30. vii.	—	6	—	—	—	—	4. viii.	
14	26. vii.	29-30. vii.	—	7	—	—	—	—	4. viii.	
15	26. vii.	29-30. vii.	—	7	—	—	—	4	4	
16	28. vii.	31. vii.	—	—	—	—	—	—	27. viii.	
17	28. vii.	31. vii.	—	5*	—	—	—	—	>7. viii.	
18	28. vii.	31. vii.	—	7	—	—	1	1	2	} Found as thrown-off buds.
19	3. viii.	7. viii.	—	5	—	—	—	—	>7. viii.	$G_3$ —2 $P$ .
20	5. viii.	8-15. viii.	—	6	—	—	—	—	19. viii.	
21	7. viii.	8-15. viii.	—	5	—	—	—	—	24. viii.	
22	7. viii.	8-15. viii.	—	4	—	—	—	—	24. viii.	
22 bis	7. viii.	8-15. viii.	—	8	—	—	—	—	24. viii.	
23	16. viii.	19. viii.	—	6	—	—	—	—	24. viii.	
24	16. viii.	20. viii.	—	6	—	—	—	—	24. viii.	

A week's interruption in the investigation.

Dissolved.

TABLE IV (continued)

$G_3$	Date of appearance	Date of casting off	Number of tentacles, Type:			Begin-ning of budding ( $G_3$ )	Number of buds of $G_3$			Remarks
			$P$	$PH$	$\gamma$		$P$	$PH$	$\gamma$	
$B\ 17, 25$	17. viii.	20. viii.	6	—	—	22. viii.	—	—	—	Perished
$B\ 26$	17. viii.	21. viii.	—	8	—	—	—	—	—	24. viii.
$B\ 27$	18. viii.	21. viii.	—	7	—	—	—	—	—	24. viii.
$B\ 28$	19. viii.	22-24. viii.	—	6	—	—	—	—	—	24. viii.
$B\ 29$	19. viii.	22-24. viii.	—	5	—	—	—	—	—	24. viii.
$B\ 30$	21. viii.	22-24. viii.	—	—	—	—	—	—	—	24. viii.
$B\ 31$	21. viii.	—	—	5	—	—	—	—	—	4. ix.
$B\ 32$	23. viii.	27. viii.	5	—	—	—	—	—	—	2. ix.
$B\ 33$	25. viii.	29-1. ix.	—	6*	—	—	—	—	—	2. ix.
$B\ 34$	26. viii.	29-1. ix.	—	6	—	—	—	—	—	2. ix.
$B\ 35$	27. viii.	29-1. ix.	4	—	—	—	—	—	—	2. ix.
$B\ 36$	28. viii.	29-1. ix.	—	—	—	—	—	—	—	2. ix.
$B\ 37$	29. viii.	2. ix.	—	6	—	—	—	—	—	2. ix.
$B\ 38$	29. viii.	2. ix.	—	7	—	—	—	—	—	2. ix.
$B\ 39$	1. ix.	4. ix.	—	6	—	—	—	—	—	4. ix.
$B\ 40$	1. ix.	4. ix.	6	—	—	—	—	—	—	4. ix.
Line $B\ 17$	—	—	12	25	4	$G_3$	7	21	13	—
$B\ 20\ 1$	22. vii.	25. vii.	6	—	—	25. vii.	—	—	—	31. vii.
$B\ 20\ 2$	23. vii.	25. vii.	—	8	—	—	—	—	—	> 7. viii.
$B\ 20\ 3$	24. vii.	26. vii.	—	—	—	—	—	—	—	> 7. viii.
$B\ 20\ 4$	24. vii.	26. vii.	—	8	—	—	—	—	—	—
$B\ 20\ 5$	24. vii.	26. vii.	6	—	6	—	—	—	—	—
$B\ 20\ 6$	24. vii.	28. vii.	—	7	—	—	—	—	—	5. viii.
$B\ 20\ 7$	24. vii.	28. vii.	5	—	—	—	—	—	—	—
$B\ 20\ 8$	25. vii.	29. vii.	—	7	—	—	—	—	—	5. viii.
$B\ 20\ 9$	25. vii.	29. vii.	—	3	—	—	—	—	—	5. viii.
$B\ 20\ 10$	26. vii.	30. vii.	5	—	—	—	—	—	—	—
$B\ 20\ 11$	27. vii.	31. vii.	—	6	—	—	—	—	—	5. viii.
$B\ 20\ 12$	28. vii.	4. viii.	5	—	—	—	—	—	—	5. viii.
$B\ 20\ 13$	29. vii.	4. viii.	—	6	—	—	—	—	—	5. viii.
$B\ 20\ 14$	?	20. viii.	—	—	5	—	—	—	—	5. viii.
$B\ 20\ 15$	?	20. viii.	—	—	5	—	—	—	—	—
Line $B\ 20$	—	—	5	6	4	$G_3$	—	—	—	—

$B\ 17$ —Preserved on 4. ix. with its two buds  $B\ 31$  and  $B\ 38$ .

{ Opposite position.  $G_3$ —6  $P$ ;  $G_4$ —2  $P$ .

$G_3$ — $P$ .

On 31. vii. two forked tentacles.

In vertical with  $B\ 1$  and  $B\ 2$ ; lower than  $B\ 3$ .

31. vii. two tentacles stuck together.

Monstrous Hydra.

Depression. All capsules have disappeared from the tentacles and body.

Dissolved.

Line  $B\ 20$

TABLE IV (continued).

$G_3$	Date of appearance	Date of casting off	Number of tentacles. Type:				Begin- ning of budding ( $G_3$ )	Number of buds of $G_3$			Remarks
			$P$	$PH$	$?$			$P$	$PH$	$?$	
$G_3$											
B 21.1	22. vii.	25. vii.	5	—	—	—	—	—	—	—	5. viii.
2	23. vii.	25. vii.	4	—	—	—	—	—	—	—	5. viii.
3	24. vii.	26. vii.	6	—	—	—	—	—	—	—	27. vii.
4	24. vii.	26. vii.	4	—	—	—	—	—	—	—	27. vii.
5	25. vii.	27. vii.	—	7	—	—	—	—	—	—	5. viii.
6	25. vii.	27. vii.	4	—	—	—	—	—	—	—	27. vii.
7	25. vii.	27. vii.	5	—	—	—	—	—	—	—	27. vii.
8	26. vii.	28-29. vii.	—	7*	—	—	—	—	—	—	27. vii.
9	26. vii.	28-29. vii.	4	—	—	—	—	—	—	—	31. vii.
10	27. vii.	30-31. vii.	5	—	—	—	—	—	—	—	31. vii.
11	27. vii.	30-31. vii.	5	—	—	—	—	—	—	—	—
12	31. vii.	?	4	—	—	—	—	—	—	—	31. vii.
13	31. vii.	?	—	—	?	—	—	—	—	—	—
14-23	—	—	—	—	10	—	—	—	—	—	—
Line B 21	—	—	10	2	11	$G_3$	—	—	—	—	—
B 34.1	3. viii.	—	5	—	—	—	—	—	—	—	7. viii.
2	3. viii.	—	—	5	—	—	—	—	—	—	7. viii.
Line B 34	—	—	1	1	—	$G_3$	—	—	—	—	—
B 35.1-7	?	?	—	—	—	—	—	—	—	—	24. viii.
Line B 35	—	—	1	6	—	—	—	—	—	—	—
B 41.1-3	?	?	—	—	—	—	—	—	—	—	24. viii.
Line B 41	—	—	—	3	—	—	—	—	—	—	—
Line B 54	28. viii.	—	—	1	—	—	—	—	—	—	2. ix.
$G_2$	—	—	32	63	21	$G_3$	28	80	28	—	—

In the first moment doubts about the origin of this bud.

Till 5. viii. gave 5 buds with 6, 6, 6, 4 and ? tentacles. (Preserved, nature not established.) Till 24. viii.—5 buds with 7, 6, 6, 5 tentacles—dissolved.

Disappeared after 7. viii.

$G_3$ ; P—6 tent. (added to Cult. XV); PH—5, 6, 5, ? tent.—added to Cult. XXV; PH\*—7, 6 tent.—dissolved.

$G_3$ ; PH—7, 6, 5 tent. Added to Cult. XXV.

buds *P* began to form buds of the new generation on the very same day, and were able to throw off nearly a dozen in a few days—the buds *PH* generally began their budding only after some days. It was an easy matter to obtain two generations within a week from the buds *P*, while the *PH* buds often gave only one in all. Besides this, a large proportion of the *PH* buds showed small dimensions, absorbed food badly, and died off before it was possible to result in germination. It is notable that I did not once get true *H*.

The third generation went through its vegetative segregation in the same way as the second. Along most of the lines *PH* prevailed over *P*. And, as before, the *oligactis* with one long tentacle (*PH\**) were more capable of living than the true *PH*; and in some lines allowed *P* to have preponderance over *PH* in their posterity. Without going into the details of the segregating process I should like to point out a few lines which attracted my attention for one reason or other.

*Line B 2.2, 13. vii.* (Genealogical Table II), illustrates the segregation of an oligactoid of the type *PH\**. Pl. XVI, figs. 64 and 65, clearly represent the aspect which pertained to oligactoids of this type. The tentacle which made itself prominent by its length, was clearly distinguishable from the rest which surrounded the hypostome. This difference was clearly marked at the very first commencement of their growth, as can be distinctly seen on bud *B 2.2.1*. The difference between the tentacles was kept up a considerable time. On this *Hydra* it was noticeable over a fortnight. Usually the difference in the dimensions passed off by degrees and all the tentacles became similar. But occasionally we noticed an unusual lengthening of one of the habitually short feelers.

Pl. XVI, fig. 65, pictures the same oligactoid at the instant of segregation. Highly characteristic was the germination of the tentacles on the buds of the third generation. On the hypostome of *B 6* (= *B 2.2.6*) there appeared *nine* tentacles at once! while on the top of bud *B 7* there had appeared but four tentacles characteristic of type *P*.

Before the eighth bud was thrown off, the preparation became depressed. The result of this was that bud *B 8* was kept back in the budding zone, and, as soon as the depression was over, passed on to the stalk (Pl. XVI, fig. 66). But as by this time the bud had grown to considerable dimensions, the preparation bore in its outward aspect a resemblance to the pictures of the “longitudinal splitting” of *Hydras*, a phenomenon which evidently does not occur in nature.

Pl. XVI, fig. 67, shows a further stage in the development of the process of parting asunder. Each *Hydra* separated its budding zone—the fundamental line (*B 2.2*) continued to give off buds (to segregate):

$B\ 9$ (= $B\ 2.2.9$ )	= $PH^*$ (with 5 tentacles);
10	= $PH^*$ (5 tentacles);
11	= $PH$ (with 9 tentacles!);
12	= $PH$ ( „ 5 „ );
13	= $PH$ ( „ 5 „ ), etc.

The bud  $B\ 8$  (= a line of the third generation =  $B\ 2.2.8$ ) while still sitting fast on its maternal organism began to throw off buds of the fourth generation:

$B\ 1$ (= $B\ 2.2.8.1$ )	= $P$ (4 tentacles);
2	= $P$ (4 „ );
3	= $P$ (4 „ );
4	= $P$ (4 „ ), etc.

It is remarkable that notwithstanding the abdominal parts of the parental organism ( $B\ 2.2$ ) and its bud ( $B\ 2.2.8$ ) being united by a common stalk and the alimentary canal, they differed all along in the tints of the body colour.

The components parted definitely only at the end of a fortnight after the stage depicted in Pl. XVI, fig. 67. Thus it happened that the fundamental, ancestral oligactoid of  $G_2$  saw its posterity of  $G_4$  still clinging tight to its body!

*Line  $B\ 2.2$*  produced during six weeks of its lifetime on the whole the following:

$$G_3 = HP : 17 PH.$$

*Line  $B\ 2.5$*  (17. vii.) interests us through my having mistaken the bud from which I developed this line, on its first appearance for a  $P$ , although I even then made a note (on p. 316) of the somewhat unusual index of tentacles:  $1 > 2 > 3 > 4$  instead of  $1 = 2 > 3 \geq 4$  (Pl. XVI, fig. 59). This difference in the dimensions disappeared, however, in time, and I went on developing the line, taking the fundamental organism for  $P$ .

My astonishment was therefore great when the second bud of the third generation ( $G_3 = B\ 2.5.2$ ) bore the type  $PH$ . This bud grew up with seven short straight tentacles. The foundation of the tentacles of the other buds showed also several irregularities, as seen in Pl. XVI, fig. 68, which represents the segregation of the original individual of the third generation.

The sum total of new organisms produced by  $G_3$  is as follows:

$$11 P : 8 PH : 5 ?.$$

Such a large number of  $P$  individuals, and of individuals of no



definable type, is a sign that when the bud  $G_2 = B\ 2.5$  was in formation there entered into its organism a considerable number of the elements of  $P$ . It is characteristic that even the increase in the number of tentacles from four to six presented nothing extraordinary for this type (see the diagram at the top of Pl. XVI, fig. 68). I therefore supposed at first that I was dealing, in this case, with the segregation of a "recessive" form, if this term may be applied to cytomictical chimaeras at all.

*Line B 4.1*, type  $PH^*$ , 18. vii. (Genealogical Table III). Out of this line I have chosen a phase of very lively segregation for my illustration (Pl. XVI, fig. 69). Upon this Hydra there are to be seen six buds of the third generation of all types:

$P$  ( $B\ 4$  and  $7$ );  $PH$  ( $B\ 5$  and  $8$ );  $PH^*$  ( $B\ 6$ ); and ? ( $B\ 9$ ).

The Hydra itself belonged to the type  $PH^*$ , which can still be seen by the uncommon length of one of its feelers<sup>1</sup>. In the course of its individual life the number of tentacles increased to nine, a very unusual thing for true *oligactis*; it then decreased again to eight.

During the one month of its existence this line brought forth:

$$G_3 = 4\ P : 25\ PH : 7\ ?.$$

Out of the life-course of *Line B 4.2* (type  $PH$ ) I portray the moment which illustrates a very regular, alternating opposite position of six buds: all the buds of the third generation  $G_3$ ,  $B\ 1$ — $B\ 6$  are sitting cross-wise, relatively to the longitudinal axis of the animal (Pl. XVI, fig. 70).

$G_3$  produced in this line:

$$2\ P : 9\ PH : 3\ ?.$$

*Line B 17.1*, type  $PH^*$ , 21. vii. (Genealogical Table IV), is interesting because it did not produce a single individual of type  $P$ . The whole  $G_3$  consisted in this line of  $PH$ :

$$G_3 = 0\ P : 7\ PH : 0\ ?.$$

The number of individuals not being large, we cannot speak of the constancy of type  $PH$ . Yet down to this Hydra we found nowhere such constancy in the manner of budding. The only exception was in the segregation of the original chimaera  $PCh$ , which throw off at a time eight and nine buds of the type  $PH$ , without alternating them with buds of the type  $P$ .

<sup>1</sup> In my preliminary note (*Biologisches Zentralblatt*, 43) I refer the individual to  $G_1$  and its buds to  $G_2$ , starting from the stock oligactoid  $B\ 4$ , as being the parental organism.

The stock Hydra of *Line B* 17.5, 27. vii., gave rise to suspicion because, in its behaviour towards one or other of the types, it most resembled *P*, but *two* of its tentacles showed an extra length. For this reason I reluctantly referred it to type *PH\*\**. It generated

$$G_3 = 6 P : 1 PH : 2?.$$

I have refrained from giving the history of the other lines, because their segregation differed in nothing from the usual course of these processes.

Summing up  $G_3$  in all its lines, we get:

$$28 P : 80 PH : 28? = 1 : 2.86.$$

If, however, we exclude the doubtful line *B* 17.5 we have:

$$21 P : 76 PH = 1 : 3.6.$$

TABLE V.

Type of buds	Number of tentacles								Number of buds
	?	3	4	5	6	7	8	9	
<i>P</i>	2	2	9	7	8	—	—	—	28
<i>PH*</i>	—	—	—	10	12	1	—	—	23
<i>PH</i>	1	—	—	8	23	17	6	2	57
?	6	—	7	8	7	—	—	—	28
$G_3$	9	2	16	33	50	18	6	2	136

## 12. The Fourth Generation. $G_4$ .

This generation produced the maximum number of buds, viz. 153, but it failed to add to the stock of interesting details or peculiarities concerning the budding process.

There is one fact, however, which becomes clear as soon as we study the genealogical tables. I developed my generations only along the lines of oligactoids (*PH*) which had kept their original character through three generations. Nevertheless, they had not lost their cytomitical nature and went on segregating off the same true individual receders (*P*) as before. This phenomenon reminds one of the "hybrid atavism"; it tells us that the bearer of the characters of *P* possess the faculty of keeping intact, in a latent state, through a whole series of generations their innermost constitution, so as to reappear in their true original genotypical purity, whenever a corresponding bud-germ becomes active.

Among other things I have shown in Pl. XVI, fig. 71, a whole series of individuals of the generation  $G_3$ , which had been set aside in order to germinate buds of  $G_4$ : *B* 9, 10, 11, 13, 14 and 16. These all belonged to the

same line; and were thrown off one after the other by the same individual of the second generation ( $G_2 = B\ 4.1$ ), but they all varied greatly in size. Only the largest of them— $B\ 4.1.13$ —put forth any buds, the others all dying off without bringing forth any.

In the following tables I have given only the totals of all the sums of the separate lines:

Line  $B\ 2.2.1$ , type  $PH^* - 3\ P : 8\ PH : 1?$   
 „  $2.2.9$ , „  $PH^* - 3\ P : 13\ PH : 2?$   
 „  $2.5.6$ , „  $PH - 2\ P : 5\ PH : 5?$

The whole posterity of Line  $B\ 2$  with all the other buds not counted here, gave the sum of:

$8\ P : 33\ PH : 9?$  ( $1:4.13$ ).

In the posterity of Line  $B\ 4$  we find:

Line  $B\ 4.1.13$ , type  $PH^* - 6\ P : 13\ PH : 2?$   
 „  $4.1.21$ , „  $PH - 1\ P : 10\ PH : 1?$   
 „  $4.1.22$ , „  $PH^* - 2\ P : 9\ PH : 1?$   
 „  $4.2.4$ , „  $PH^* - 0\ P : 11\ PH : 6?$   
 „  $4.2.5$ , „  $PH^* - 0\ P : 7\ PH : 2?$

The total  $G_4$  of this line comprised:

$10\ P : 51\ PH : 14?$  ( $1:5.10$ ).

The posterity of Line  $B\ 17$ :

$4\ P : 21\ PH : 3?$  ( $1:5.24$ ).

The sum total of the fourth generation of the above three lines showed:

$22\ P : 105\ PH : 26?$  (i.e.  $1:4.8$ ).

This proportion shows clearly the increase in the number of oligactoids with each succeeding generation.

TABLE VI.

Type of buds	Number of tentacles								Number of buds
	?	3	4	5	6	7	8	9	
$P$	—	—	6	7	9	—	—	—	22
$PH^*$	—	—	1	6	6	1	—	—	14
$PH$	—	—	—	14	39	33	3	2	91
?	13	1	5	1	6	—	—	—	26
$G_4$	13	1	12	28	60	34	3	2	153

13. *The Fifth Generation.  $G_5$ .*

The tables of the fourth generation show that we limited our numerical investigations to the posterity of three lines, *B* 2, *B* 4 and *B* 17, which we had set apart from the first generation. Nevertheless, the number of individual organisms had grown so much that the daily control of all organisms under cultivation was almost impossible, and I had to investigate them every second or even third day.

I do not give detailed extracts from all my Records, as my observations grew inevitably less interesting; I must, therefore, refer the reader to the genealogical tables (II–IV) which show far better than long lists of dry figures could do, the process of segregation. (Example, Pl. XVII, fig. 72.)

In most of the lines the proportion of *PH* to the segregating *P* buds remained the same. On a par with this it was interesting to note that along some of the lines, e.g. *B* 4.2.4.3—a pure *PH*—all the individuals of the fifth generation developed after the same type *PH*:

$$0 P : 9 PH : 0?$$

Only the line *B* 17.1.1.3 showed unexpectedly a predominance of *P* = 5 *P* : 6 *PH*—and this proportion had an immediate influence upon the sum total of results.

The general proportion between the various types of buds of the whole  $G_5$  was the following:

$$20 P : 71 PH : 14? \quad (1 P : 3.55 PH).$$

TABLE VII.

Type of buds	Number of tentacles								Number of buds
	?	3	4	5	6	7	8	9	
<i>P</i>	—	—	5	5	10	—	—	—	20
<i>PH</i> *	—	—	—	3	5	—	—	—	8
<i>PH</i>	1	—	—	1	32	25	2	2	63
?	6	—	2	4	2	—	—	—	14
$G_5$	7	—	7	13	49	25	2	2	105

*The Sixth Generation.  $G_6$ .*

In this generation a remarkable preponderance of buds of the type *PH* became evident. In a whole series of lines not a single bud *P* was segregated. This was especially the case with the line *B* 2.2.1.6.7, where all the  $G_5$  out of the fifteen buds consisted of oligactoids only:

$$0 P : 15 PH : 0?$$

In the other lines oligactoids varied between 1:3.3 and 1:6 and 1:10.

On the whole  $G_6$  consisted of:

$$7 P : 72 PH : 7? \text{ or } 1 P : 10.29 PH.$$

TABLE VIII.

Type of buds	Number of tentacles								Number of buds
	?	3	4	5	6	7	8	9	
$P$	—	—	2	2	3	—	—	—	7
$PH^*$	—	—	—	6	6	1	—	—	13
$PH$	1	—	—	5	31	20	2	—	59
?	1	—	—	3	3	—	—	—	7
$G_6$	2	—	2	16	43	21	2	—	86

*The Seventh Generation.  $G_7$ .*

The lateness of the season and my forced departure from Petrograd obliged me to close my Hydra investigation just as the seventh generation was beginning to germinate. In the short space of time that intervened between the last week in August and my departure the first week in September, I was able to collect only twenty-eight buds, which were distributed as follows:

$$3 P : 20 PH : 5? \text{ or } 1 P : 6.67 PH.$$

I wish to draw attention to a few *oligactis* which were thrown off along those lines that had been for six generations typical oligactoids. Six vegetative generations separated these Hydras from the old stock Hydra *oligactis* which I had used for my insertion experiment. Throughout the whole of this long period (about two months) the *I*-cells of the *P* type being passed on from generation to generation, had preserved their genotypical purity perfectly, and had at last produced "atavistic" buds.

This circumstance proves the unusual tenacity of the genotype. Notwithstanding their close proximity (a graft-parabiase) the cells exerted evidently no modifying influence upon one another (in compliance with the requirements of the theory of chimerical organisms).

Upon comparing the results of all our investigations, we get the following table for the budding process:

TABLE IX.

	$P$	$PH$	Proportion
$G_1$	16	44	1:2.75
$G_2$	32	63	1:1.94
$G_3$	28	80	1:2.86 (1:3.6)
$G_4$	22	105	1:4.80
$G_5$	20	71	1:3.55
$G_6$	7	72	1:10.29
$G_7$	3	20	1:6.67

Which makes in the sum total, the mean:

$$128 P : 455 PH \text{ or } 1 P : 3.55 PH.$$

To this sum must be added 106 buds of an indefinite character, so that the total of buds got from the stock cytometical chimaera and which passed through my hands reaches 689 specimens. I have not included in this sum those individuals got from true *oligactis*, whose number also makes up several hundreds, but which I generally threw aside at the very beginning of their generating act.

During artificial selection all the individuals of the true *P* type were cast aside and finished off; oligactoids alone were allowed to generate. Thanks to this, I had such a large collection of them towards the close of summer, that I was able to arrange separate cultures of them. Yet I could not keep these cultures absolutely pure for long. From time to time there were thrown off true *oligactis* buds, which increased so rapidly that they out-numbered my hybrids, and in the process of *natural* selection sometimes even entirely supplanted the oligactoids. I have not made many observations in that line yet. I attempted putting occasionally oligactoids into a natural water-reservoir, but I have not yet succeeded in verifying the results of these experiments.

Looking at the above table we find that the number of oligactoids grows uninterruptedly, reaching in the  $G_6$  the proportion 10:1.

The gradual decrease in the number of segregated *oligactis* buds made me suppose that a gradual clearing out of *I*-cells of the *oligactis* type from the bodies of oligactoids was taking place. This clearing process will become specially clear if we look not at the results of parallel additions of separate generations, but at the genealogy of separate lines, which have been developed from *B* 2, *B* 4 and *B* 17. I found it, however, impossible to obtain a perfect "clearing out" of the *P* elements from the oligactoids.

In spite of this, the inconstant, segregating, "heterozygous" oligactoids showed an uninterrupted disposition throughout all the generations to pass into a stable, "homozygous" state—the condition of constant, outwardly intermediate, and by descent—*vegetative* hybrids.

If such a transformation had really taken place, and if the oligactoids had kept their characters even during sexual generation, then we might have proved satisfactorily the possibility of obtaining new "species" in a purely vegetative manner, without the sexual elements taking any part in it whatever.

I shall be obliged to return to this question once more in my concluding section.

In conclusion I have drawn up a table to review the number of tentacles with which buds were cast off the maternal oligactoids during their segregation. The review has been drawn up according to the customary statistical methods. A comparison of the tables for *P* buds and *PH*, shows at once the sharp difference in the number of *oligactis* tentacles and oligactoid ones. The type *PH\** has been treated under a separate head, but, as can easily be seen, it differs hardly from the true *PH* type. Among the buds of an indefinite nature (?) one-third (33) belongs to those buds which had only shown a germ without being thrown off, and which then died, together with the maternal organisms, on which they were bred. The rest of the buds had been individuated, but it was not possible to determine their type, as they had not begun to germinate, while the very first moments of their development had escaped observation.

TABLE X.

*PCh.* 164.  $G_1-G_7$ 

Type of buds	Number of tentacles								Number of buds
	?	3	4	5	6	7	8	9	
<i>P</i>	3	2	39	45	39	—	—	—	128
<i>PH*</i>	—	—	3	37	34	6	—	—	78
<i>PH</i>	5	—	—	49	174	122	18	7	377
?	33	2	18	23	29	1	—	—	106
$G_1-G_7$	41	4	60	154	276	129	18	7	689

#### 14. *The Segregation of Other Cytomictical Chimaeras.*

All the above-mentioned results and summaries relate to the vast posterity which was developed out of *one* stock parent chimaera.

One might fancy that this phenomenon stands alone and was noticed accidentally in this one experiment.

That such is not the case has been proved by the following observations.

Whatever the origin of the cytomictical chimaera, whatever quantity of red substance was necessary for its formation, it always began segregation. And during this budding process it always produced only *oligactis* and oligactoids. Such segregation I observed in whole series of experiments, but the close investigation of *all* the generating chimaeras was beyond my unaided powers. I shall mention now only a few of these experiments.

1. Exp. 170, June 28th, 1922. Complantation. Genealogical Table V. This preparation did not begin budding at once. It passed temporarily

into a state of depression, and upon emerging from it, there was not a trace visible in it of the elements of the red Hydra.

Only on July 21st, nearly a month later, did it begin to bud, and began at once to develop oligactoids. A cursory glance at Pl. IX, fig. 73, which illustrates the commencement of the budding process, shows that we are dealing here with a phenomenon similar to the one analyzed in the preceding experiment. We recognize the same typical oligactoids, true *PH* and *PH\**.

The *oligactis* type was segregated off for the first time by *B* 13 and appeared after that only in single specimens.

The whole of  $G_1$  consisted of 44 buds, grouped as follows:

$$8 P : 32 PH : 4?.$$

This experiment made manifest the prevalence of *PH* over *P* even in first generation; to every *P* there were 4 *PH*. Evidently more of the red element had entered the substance of the parent organism than was the case in Exp. 164. Then also the budding went on much quicker, 44 buds having begun to germinate in the time from July 21st to September 3rd.

The first generation of oligactoids went through the usual segregation. Pl. XVII, figs. 74 and 75, show an oligactoid, *B* 2 (which is also shown in Fig. 73), at the moment of its segregating into *PH* (*B* 2.1) and *PH\** (*B* 2.2). The same Hydra is exhibited in an expanded and in a contracted state. The first of the figures (No. 74) shows how the tentacle which is distinguished by specially great length has kept its proportionate size.

I have given neither Records from my Journal nor accounts of the segregating process. All these details are sufficiently clearly shown in the appended genealogical table (V).

The summary of all the generations recounts the following results:

$$\begin{aligned} G_1 &= 8 P : 32 PH : 4?—1 P : 4 PH; \\ G_2 &= 15 P : 60 PH : 13?—1 P : 4 PH; \\ G_3 &= 6 P : 17 PH : 3?—1 P : 2-83 PH. \end{aligned}$$

Sum total:

$$29 P : 109 PH : 20? \text{ or } 1 P : 3-76 PH.$$

This unexpected increase in the number of *P* in  $G_3$  was brought about by Line *B* 8.2, which gave the segregation:

$$5 P : 7 PH : 1?.$$

Without it the proportion of *P* to *PH* in  $G_3$  would have been as 1:10. On the whole the figures of the given experiment were not very high.



In any case we must affirm that the enriching of those generations with oligactoids went on quicker than during the former experiment.

But in the sum total of all buds of the three generations (Exp. 157) the proportion of  $P$  to  $PH = 1 : 3.76$  showed little deviation from that of the previous experiment ( $1 : 3.55$ ).

2. Exp. 168 has been already described by me as an example of the regulation process after the insertion of *oligactis* into *vulgaris*. I have already spoken of the unusual character of the budding.

The number of buds which I have collected is too insignificant. I mention it only in order to show how inconstant the numerical proportions are in different cases of segregation, and how subordinate their importance is (Genealogical Table VI).

$G_1$  produced 1  $P : 4 PH : 1 ?$

$G_2$  „ 1  $P : 8 PH : 2 ?$

$G_3$  „ 0  $P : 4 PH : 1 ?$ .

All in all buds: 2  $P : 16 PH : 4 ?$  or 1  $P : 8 PH$ .

The segregation of the cytomitical chimaera  $PCh$  168 brought fresh and interesting colouring into the rather monotonous budding picture.

I had been interested for some time in the question whether the influence of the chemical reaction of the component parts upon each other did not play some part in the segregation; or whether it was entirely based on some definite material substratum. Although I was really convinced of the correctness of the second supposition, yet some doubts might possibly arise. One incident in the life of one of these chimaeras gave a clear answer to this question also.

The germs of the first two buds appeared very close to each other (Pl. XIII, fig. 36). In my Diary I made a note that they would probably both be thrown off by the common base. This turned out to be the case. Both buds were cast off in the form of a double-headed Hydra with rather a deep incision between the two heads.

It is usual to quote such two-headed Hydras in proof of the existence of a longitudinal parting in Hydras. Boecker (1914) already has proved the absolute incorrectness of this opinion, and my last investigation adds a fresh support to his proof.

One of the buds that produced the double-headed Hydra was a true *oligactis* ( $B1 = B4$  on Pl. XIII, fig. 36), the second, an oligactoid ( $B2 = B5$  on Pl. XII, fig. 35). This circumstance had a quickening influence upon the fate of the preparation.

The longitudinal fissure having individuated both Hydras, gave to

each its own budding zone. The oligactoid *B* 2 immediately began segregation, while the left component, *B* 1, began to develop pure *P*'s. Pl. XVII, fig. 76, has caught just the moment.

Oligactoid *B* 2 produced consecutively the buds:

- B* 1.1—*PH*, 5 tentacles;
- 1.2—*P*, 7 tentacles;
- 1.3—*PH*, 6 tentacles, etc.

In all:

1 *P* : 6 *PH* : 2?

*Oligactis B* 1 produced 8 buds—all true *P*.

The uninterrupted connexion which the common canalization system had established between the two components, would certainly have made any chemical reaction possible, if there had been any at all. Yet, in spite of the long union of the two components by means of the abdominal cavities, the component *P* showed no change whatever as a result of the close proximity of its neighbours of the oligactoid type. (This peculiarity could be also observed during the regulating process of the original preparation of Exp. 168.)

The subsequent slow parting asunder of the two components presented nothing of particular interest. It proceeded as every ordinary, so-called "longitudinal" splitting of Hydras does proceed, so that I need scarcely stop over it. These buds had been split off on August 2nd; but they did not part company before August 27th.

3. In conclusion I should like to say something more of the budding of my first cytomictical chimaera of the year 1921, which I have already spoken of in this Essay, as an example of the course "regulation" takes under complantation (Exp. 105, Genealogical Table VII).

Translating last year's notes into the language of this year's investigations, and looking over once more the Records and sketches, I find that my attention had already then been attracted by the strange manner of budding. I had obtained in 1921 nine individuals of the first generation, which were all evidently specimens of *PH* and *PH*\* (of the latter there were three individuals).

In the second generation eleven Hydras were generated whose type I was unable to determine, as the depression which took place in 1921 blurred the picture considerably, while the absence of *P* buds made it impossible to picture any kind of segregation. During this period of depression several buds were split off with two, and even three heads, remaining a long time in this state. They could furnish no answer to the question as to the essential nature of the budding act.

15. *Sexual Generation of Oligactoids (?)*.

In the autumn of 1922, on my return to Petrograd, I found in one of the vessels where there had been oligactoids previously a nest of eggs. The oligactoids themselves had died. The eggs lay round an open central field, and were sticking together in a shapeless mass. The aspect of the eggs reminded one of the species *oligactis*, but the embryotheca differed—it had no spines, but was rather smooth.

Towards Christmas time about ten Hydras were hatched from these eggs, all having the appearance of *oligactis*. The structure and character of the capsules (cnidocysts) showed many peculiarities. Unfortunately these Hydras did not pass on to budding. They all developed one or two testes, after which they passed into a singular state of depression: they threw out all their capsules from the tentacles and from the walls of their bodies, ceased taking food, vacuolated their bodies and then perished.

Not knowing exactly whence these eggs originated I refrain from expressing a definite opinion about the sexual generation of oligactoids.

## GENERAL CONCLUSIONS.

1. *Organic Regulations*. The main interest of the fundamental grafting experiments lies in the phenomena of organic regulations which accompany them. We should therefore refer all experiments for obtaining chimaeras to the mechanics of development, or more strictly, to the physiology of form-shaping. This statement has been most accurately illustrated by the brilliant experiments on the embryos of *Triton* by Spemann. Other originators of chimaeras (Winkler, Schaxel) also see in this the principal significance of chimaeras.

In connexion with the problem of organic regulation, I should like to place foremost the question of the so-called *organizing centres*, of those material sources of the form-determinating stimuli, which awaken in a manner still unknown to us, the sleeping possibilities of the indifferent cells, and draw them forth to the surface.

Frequently this new activity of the germs produces an impression as if the tissues or the organ had been entirely reconstructed, and replaced by a new "heteromorphic" creation.

We have also seen that the influence of such centres was absolute, automatic. Every substance which comes within their field of action, be it of their own species, or of a strange one, was necessarily subjected to reconstruction.

This automatic way of proceeding is clearly often misplaced, and from the point of view of the species, even hurtful. We saw how the red hypostome called forth new tentacles in the neighbouring brown substance; how these tentacles formed a new brown hypostome which absorbed the red one and finally the whole Hydra which had given it life!

This makes one involuntarily venture to put forth a theory on the chimerical nature of "form-determining irritations."

The second place will be occupied by the question of the organic regulation in general.

In our experiments on Hydras we come across that direction taken by processes which Driesch has called "equifinal." This equifinality showed itself in our experiments in the results obtained from the regulating processes, which became active in the hypostome and the tentacles during the heteroplastic grafting of two whole Hydras. Whatever the mutual orientation of the components, however many tentacles might become united in the common hypostome, the end result of this complicated preparation—sometimes by means strange and grotesque—was a Hydra the outer aspect of which corresponded in every way to the standard description of a typical, six-tentacled stalked Hydra (*oligactis*).

But if we turn our attention to the essence of these processes, we shall find that the result of all kinds of operations is *always* an organism which has been called a cytomictical chimaera.

I shall not stay to explain the causes of the regulating processes which change a complex preparation into a common Hydra, as I have tried to solve these problems in a separate Essay ("Studies on organic regulations"). Nor shall I stop to speak of the innermost nature of the phenomenon of parabiasis in brown and red *I*-cells within the common layer. I hope to examine this question in that part of my work which I have devoted to microscopical investigations. Yet it is perhaps needful to say that I have approached both questions from a distinctly material basis.

It seems to me that it is possible to look at the equifinality of the regulating processes as at a specific counter-reaction of the living substance of a Hydra to all manner of external stimuli. A non-fertilized egg has but one specific answer to every stimulus—development. The inner balance of tissues is brought about by the original parabiasis of the *I*-cells.

2. *Chimericality*. I have said already that genetics can profit but little from chimaeras. Nevertheless, the little that chimaeras give is very valuable. Chimeric preparations point to the tenacity of what we call genotypical constitution of the organism. This was particularly brought

out and emphasized by Schaxel. My own experiments have confirmed it anew.

In cytomictical chimaeras the *I*-cells of two species of Hydras are closely united in one common layer. They are drawn into all the physiological processes that their organism undergoes. They are under the immediate influence of the nutrient fluids and nervous stimuli of the home-organism. They pass through a series of six vegetative generations of oligactoids in a latent state. Being activated in the seventh generation, they give true *oligactis*, which continue their propagation without any further segregation.

The principle of chimerality is thus kept inviolate; the cells of two species lie side by side, but exert no mutual influence whatever upon the genotypical constitution.

In connection with this phenomenon I cannot help recalling to mind the "nuclear-chimaeras" of Lotsy (1918). Renner had suggested that *Oenothera Lamarckiana* always produced two kinds of gametes—*velutina* and *laeta*. Lotsy developed this view and called these gametes "nuclei," which keep their genetic individuality in the sexual cells of the plant. N. Heribert-Nilsson (1920) has correctly observed that it would be easy to include every kind of monohybrid disjunction in this term, and that there is no necessity for inventing new terms.

But, from a certain point of view, Lotsy was right. If he had developed his opinion of a chimaera not starting from a nucleus, but from "factors" (which he evidently had in view), he would have been using the terms of modern genetics—since the whole hypothesis "of the purity of gametes" is indeed nothing but another term for "*factorial chimerality*" of the germ cells of every hybrid or every heterozygote. The factors or genes lie within it side by side but without influencing each other in the least and keeping their genotypical purity entire. The way out of this chimerical state is the well-known act of the segregation into the true homozygous forms in the second generation.

If we connect with the term "heterozygous form" the notion of "factorial chimerality" we shall get rid of the somewhat hazy expression "hypothesis of the purity of gametes."

An unbiassed investigation of the phenomena of anatomical chimerality allows me to think that the opinion I express here is not altogether improbable. Cytomictical chimaeras form an intermediate step between anatomical and nuclear chimerality (myxochimaeras of Burgeff, 1914–15). From these there is but a step to the factorial chimaeras.

The following historical reference may not be without interest:

In the year 1865 there appeared at the same time with Mendel's work, the researches of the French botanist Naudin. If we consider how Naudin understood the nature of hybrids, we shall find that his definitions coincide in an extraordinary manner with some of our modern ideas. Even the term segregation or disjunction ("*la disjonction de deux essences spécifiques dans le pollen et les ovules de l'hybride*") is used by him for the same act of parting in the second generation. Only the fact that he was working with species-hybrids and not with racial ones (which had been used by Mendel in his experiments) prevented his seeing that law-abiding order of things which goes now by the name of Mendel's law.

In Naudin's conception the sexual cells of the hybrid were just what we now propose to call "factorial chimaeras." But Naudin extended this peculiar anatomical structure to the whole of the plant as well: he ascribed to every hybrid cell a dual character. So that every hybrid was nothing but a complicated mosaic composed of the cells of both parent types, i.e. a "mosaic chimaera":

*"L'hybride serait une mosaïque vivante, dont l'œil ne discerne pas les éléments discordants tant qu'ils restent entremêlés."*

The "*disjonction*" of such a hybrid takes place most energetically in the sexual cells—and during any phase of its existence (we should say now: vegetative segregation). As an example Naudin mentions the well-known *Cytisus Adami*. We know now that that *Cytisus* is indeed a chimaera, only not a mosaic one, but a periclinal. In normal hybrids the chimerical state is only terminated by the condition of the sexual cells<sup>1</sup>.

Mendel's hypothesis "of the purity of gametes" is Naudin's "struggle of the specific substances," i.e. factorial chimericality. All these are but various characteristics of a normal heterozygous state; all this can serve to define the idea of "hybrid."

3. *Oligactoids*. In my experiments I united the living substance of two species of animal organisms into one chimerical preparation. But from the preparation this living substance issued in another form, a new one, which did not exist in nature, not identical with either of its two parental forms, but at the same time representing something intermediate between them.

Thus, by carrying the living animal through a chimerical preparation

<sup>1</sup> With greater success Naudin might have quoted as an example the well-known Andalusian hens (in the earlier explanation given by Bateson and Punnett, not by Lippincott). These hens are examples of the "vegetative segregation" of the heterozygous form in the first generation. The same can be said of other true "mosaic" hybrids.

I succeeded in synthesizing an absolutely new "species" of an animal with definite morphological characteristics that persisted through vegetative reproduction.

I called this form of the new "species" oligactoids, because its aspect reminded me most of the typical brown Hydra (*Pelmatohydra oligactis*).

From the species *oligactis* it received:

A clearly defined stalk, discernible sometimes even before disjunction, but at others becoming differentiated only on the second or third day.

A characteristic budding zone.

The capacity of throwing off buds of true *oligactis*.

The characteristic manner of contraction (during this process the oligactoid assumes the shape of a wineglass on a foot, which form is peculiar to *oligactis*).

From the species *vulgaris* it received:

The manner of sprouting tentacles on the bud (all simultaneously).

Their large number (five to nine).

Their general appearance (straight, rather short; their way of holding them in the water—in a straight cone).

The position of the buds: opposite each other, either in a verticil or irregularly.

The outer aspect of the young bud-germ (a broad cup, not a sharp cone).

Their choice of a home to stick to (at the bottom of the vessel, but not on its walls); rarely they swim head downwards clinging with the sole of their foot to the upper layer of the water.

To the completion of these characteristics there is still wanting the description of the nematocysts. But at the time when I was making my investigations of living objects, I was not yet acquainted with P. Schulze's Monograph. On my inquiry after it the author most kindly sent it me to Petrograd. But it was too late that autumn after the close of the experimental summer season, so I had to leave the question of capsules to that part of my paper which treats of microscopic research.

Besides all this, a knowledge of the structure of the sexual organs is indispensable, of the testes and the embryotheca; I am unable to speak definitely of their structure, as I am unfortunately not sure of the origin of the eggs of which I have spoken above.

However this may be, the new "species" proved absolutely persistent. I therefore trust that I have succeeded in showing the possibility of producing new forms in a purely vegetative manner. And as these forms have besides to be proved to be intermediates, and in reproduction,

constant, I fancy that my oligactoids might be called constant graft-hybrids, in spite of their segregation and their chimerical constitution<sup>1</sup>.

4. *Vegetative Segregation of the Oligactoids*. On a par with the constancy of form, the oligactoids possess one characteristic trait: they are constantly segregating.

Before speaking of the segregation of oligactoids it is necessary to consider the segregation of the stock cytomictical chimaera. All the material needed for my experiments I have taken from the posterity of one Hydra caught in the spring of 1922, which has made it possible for me to keep my line *absolutely pure*. By means of insertion and grafting I supplied new cellular elements from another species into the substance of its body.

After this the parent Hydra began to split off *oligactis* and oligactoid buds; in other words, there began vegetative segregation in the original *pure line*.

Thus we know at the present time already the following means of escape from pure lines:

(a) W. Johannsen (1908) has pointed to mutation in a pure line (bean).

(b) Protistologists (Rh. Erdmann, 1920) detect the decisive moment in the processes of parthenogenesis ("endomyxis"—*Paramoecium*).

(c) Winkler (1922) has discovered a segregation of a pure line after letting an organism (*Sol. lycopersicum gigas*) pass through a tetraploid state.

(d) Blakeslee (1922) showed that a pure line of *Datura* segregated after re-duplication of single chromosomes.

(e) And now I have found the possibility of splitting a pure line by means of passing it through a chimerical preparation (Hydra).

Returning to the segregation of oligactoids I confess that I see in this process true vegetative segregation. All the processes take place during a sexual propagation; they take their source in the chimerical nature of oligactoids. It is just this fact which allowed of my calling the oligactoids "hybrids" with the quality of "grafted" appended, which indicated the method of their creation. "Hybrid" in contemporary genetics means every heterozygous form. But in this case the name is applied not to a sex-cell, but to a whole individual. This is also the reason why I have put the term "heterozygous" everywhere in inverted commas,

<sup>1</sup> The *Oenothera Lamarckiana* is, according to Renner, a constantly segregating "complex heterozygous" plant!



so as to avoid using the term of "somatic" heterozygosis. I am unable to enter here upon the question of somatic segregation, all the more as this has been done already by Bateson in 1922.

The terms "dominants, recessives, atavism, factors," etc. are but of relative importance in connection with Hydras, but they indicate sufficiently the outward appearance of the processes and their course. The chief interest lies in the fact that all these phenomena take place during a sexual propagation and without the help of any sexual elements (about the part played by *I*-cells further on).

It seems to me that it is necessary to turn more serious attention to the phenomenon of heredity with a sexual propagation ("vegetative heredity"). There are hardly any serious systematical and well-considered works on the subject. There have been some experiments carried out with Hydras (Hanel, 1908; Lashley, 1915-16), but with poor results.

And now for numerical relations. I have said that it is hardly possible to compare these figures with the correct computations and proportions of the Mendelian segregations. These proportions depend no doubt to a great extent upon the amount of substance of the red Hydra that went to form the cytomictical chimaera (the stock oligactoid). The following facts support this view.

In the complantation experiment there was already in  $G_1$  a great majority of oligactoids (1 *P* : 4 *PH*; in insertion experiment, 1 *P* : 2.75 *PH*).

The pure *PH* did not throw off many *P*'s on the whole.

The type *PH*\* exhibited a pretty considerable diversity. Some individuals produced generations which consisted pre-eminently of *PH*; while in the posterity of others there was a predominance of *P*.

The greater mass of the posterity of the type *PH*\*\* was composed of *P*.

All these features show that the numerical proportions appear to depend on chance.

Nevertheless, we note a gradual decrease in the number of oligactoids cast off. This can only be attributed to the gradual decrease of *I*-cells of type *P* in the body of the oligactoid, i.e. with the gradual transition of the "heterozygous" form into a "homozygous" state.

5. *The Origin of Oligactoids.* The study of numerical proportions leads us directly to the question of the origin of oligactoids.

Here we are met by three possibilities:

(a) In one of the cells of type *P* or type *H*, under the influence of a neighbouring cell of secondary type, mutation had taken place. The oligactoid received its being from such a unique mutated cell, i.e.  $P \rightarrow PH$  or  $H \rightarrow HP$ . Such a possibility seems to me but little probable.

(b) The strange "fertilization" of one whole organism by a second, also whole organism, ended in the union of their *I*-cells. In this case too the oligactoid must have originated from one cell, *PH*, which would be a realisation of Winkler's idea about "Bourbons<sup>1</sup>." Unfortunately, this process cannot be proved at all.

(c) I pass on to the third possibility which seems to me the most likely. Oligactoids have their beginning in the union of separate *I*-cells, i.e.  $PH = P + H$ .

There are several considerations which speak in favour of this opinion:

The special manner of budding that Hydras have, and which, according to many authors, amounts to the activation of the germ which consists of *many I*-cells.

The existence of various kinds of oligactoids. The most constant form, and the most numerous (377 of 455) was the type *PH*. Less stable in form and numerous (78 of 455) were *PH\**; but they were more vigorous and budding more rapidly.

Type *PH\*\** approached still nearer to *oligactis*. Then come those few single individuals which I mistook at first for the segregation of "recessive" forms. It is possible that among the small dying-off oligactoids might have been found pure, "dominant" forms of the type *H*; but these forms were probably not capable of living, thanks to the liberation of some "lethal" factors while passing through the chimerical preparation.

In any case we note a whole series of gradations from *PH* to *P*. In the process of the appearance of these forms there seem to take part a great number of "multiple" factors. We can take this only for the outward expression of the mixed nature of those germs (of a group of *I*-cells) which gave birth to the development of new buds.

The principal mass of the posterity consisted, however, of pure oligactoids. This forces me to come forward with the supposition that the *I*-cells of both *vulgaris* and *oligactis* do unite in some more constant manner, and that this compound, liberating itself in the course of generations from the free cells of the *P*-type, becomes more and more constant. The simple parabiasis becomes a true symbiosis, in the "linkage" together, not of factors, but whole—potentially sexual—*I*-cells.

Only thus is it possible to explain the change of an inconstant heterozygous form into a constant and non-segregating homozygous one. (I again draw attention to Renner's theory about the *Oenothera* being a constant complex heterozygous form.)

<sup>1</sup> *Unters. üb. Pflanzbastarde*, 1912, p. 11.

6. *The Problem of the Shape of Oligactoids.* There still remains one problem unsolved—the question of the origin of the oligactoid's shape. Why the form-building action of *oligactis* cells shows itself in the general appearance of the animal and the form of the stalk, while the action of *vulgaris* is limited to the region of hypostome (type and number of tentacles) and the budding zone (the sprouting of tentacles and buds)—we are quite unable to tell. The form-building of oligactoids runs along strictly defined and perfectly law-abiding lines of its own, but we have no power at present to analyse these laws.

We are as little able either to understand the persistence of the outer shape of oligactoids in connexion with the changeableness of the inner elements which determine the outer form.

7. *The Importance of I-cells in the regulation process.* In one of his papers (issued in 1918) P. Schulze speaks of the enormous importance which the indifferent, interstitial, i.e. the *I*-cells, have in the lives of Hydras. From them arise all the various kinds of cells, nematocysts, sexual cells and germs of buds. It is they who begin and carry on the process of regeneration. In a word these *I*-cells possess the sum-total of all hereditary factors of the Hydra, i.e. in a word, they are totipotent.

In every part of the Hydra body (except in the inactive stalk and tentacles) are to be found *I*-cells. This furnishes the explanation of the great regulating power of Hydras, which bring us finally to the material substratum—to the processes that go on in the *I*-cells.

In this respect Hydras do not differ from hydroids (*Tubularia*, Driesch, 1896; *Pennaria*, Wilson, 1911; sponges, Wilson, 1907, and Müller, 1911), ascidians (*Clavellina*, Driesch, 1902, Schaxel, 1914), from planarians and some other worms—nor from any plants, i.e. from none of those organisms which possess an excellent regulating capacity. Every portion of the bodies of these organisms, having a number of totipotent *I*-cells (e.g. indifferent cells, wandering cells, archeocytes, the cells of the cambium or meristem), sufficient to keep up life in that particular portion, is capable of growing the whole organism again.

For this reason I believe that these organisms possess no “individuality”—indivisibility—as we generally understand the term. But just the reverse of this. They are all divisible, they can all be dissected into the smallest totipotent parts. I therefore suggest the term “Dividuality” for “Divisible Beings.”

And if we do, nevertheless, speak of the individuality of Hydras, plants, and such-like objects, we mean but a relative individuality, and

not one that exists in reality<sup>1</sup>. In reality there exists but divisibility—"dividuality."

Individuality—true *indivisibility*—begins simultaneously with the limitation of potential possibilities. This often takes place at the same time with the individuation of the sexual cells. Thus, in the embryonic stage, only from the moment when the embryonic cells begin to lose or limit their totipotency, does the possibility of a "division" of the embryo end, and the possibility of "indivisibility" begin. It seems to me, therefore, that during the embryonic stage of growth, side by side with the problem of the "becoming" of the organic form we can put forward the new and special problem of the "*becoming*" of the organic *individuality*.

In order to discuss the problem of individuality we must return once more to the question of organic regulation.

"Dividuality" (= divisibility) is connected in our conception of the problem with a definite condition of anatomical growth, viz. with the presence of indifferent and totipotent cells. Such are the *I*-cells of grown-up animals and separate blastomeres in the various stages of the embryonic organism. Only "divisible" organisms are capable of possessing a maximum regulation power; only in such can the regulation process have an equifinal character, precisely in the same proportion as the *I*-cell carries within it the whole fulness of hereditary germs. This detail of anatomical structure turns every isolated portion of the body into the "equipotentially harmonious system" of Driesch. It seems also to stand in correlative connexion with the "plasticity" of living matter, with the capacity for vegetative propagation, often with a sedentary mode of life and with a tendency to form colonies. I am touching here only upon separate links in the chain of ideas which I develop more fully in my "Essay on Organic Regulations."

Putting the question thus, I have tried to give the nebulous vista of organic regulation some "physical basis," which might deprive many *à priori* conceived notions of the mystical haziness that envelopes them.

Trying to explain the *origin* of the regulating processes we have said nothing as yet about the *cause* of the *outward appearance* of these processes.

The problem of the becoming of organic form—the form as such, the form *outside of functions*—is only just beginning to arouse interest. The gates leading up to this are the teaching about organizations, centres,

<sup>1</sup> W. Goetsch has contributed a whole series of papers to the magazine *Naturwissenschaftliche Wochenschrift*, 1922, on the question of the "relativity" of the idea of individuality.

and that about the form-determining stimuli, of which I have had occasion to speak already.

8. *The Soma and the Germ-plasm.* The examination of the importance of *I*-cells in the regulating processes obliges us to turn to the question on the dualism of the bodies of organisms. Weismann has carried the notion of the immortal germ-plasm and of the mortal soma far into every distant corner of the science of biology. And the extraordinary rapid development of theoretical genetics has helped much to strengthen this view.

But almost at the same time there began to be heard protestations against the fetichism which had enveloped the idea of soma and germ-plasm. Among those who lifted their voices against the idolatry of dualism, must be mentioned foremost K. A. Timiryazev.

If we take a plant, say the well-known *Begonia*, we shall quite in vain endeavour to find in it the differentiation of its body into the mortal soma and the immortal germ-plasm.

Still more naïve would be our attempt to find in plants the beginnings of "germinal track." Any somatic cell of leaf parenchyme that has kept its non-differentiated character, any cell of meristeme or cambium (= *I*-cell) can begin to sprout new leaves and shoots—and then later on, blossoms and fruit, i.e. real "germ-plasm." We see the same thing in every phenomenon of vegetative propagation, especially clear are cases of true apogamy (as Winkler (1908) means it, not Strasburger (1905)), and so forth.

It seems to me that these facts show that the teaching about the germ-plasm and the germinal track, cannot be in the least applied to plants.

The same must be said of Hydras, and of all "divisible" organisms possessing totipotent *I*-cells. We shall find no special germinal track which bears along the germ-plasm in them. The very sexual germ appears in them as diffused; in other words, "divisible" organisms have their "sexual elements" in every particle of their bodies. Every so-called non-sexual propagation must after all be called "sexual," as far as totipotent, and non-reduced *I*-cells take part in this process.

From propagation with the help of one *I*-cell we might have expected a parthenogenetic process, analogous to apogamy in plants. The investigation of the cytological details of such a process is of intense interest. Therefore it is greatly to be desired that the budding process of medusae *Margelidae* should be analyzed, as in this species (after Braem, 1908) buds take their beginning from a single cell.

Summing up my views on the theory of germ-plasm, I venture to put forward the following thesis:

(a) We observe in nature two clearly marked off poles. On the one hand stand the "divisible" organisms, such as plants, and animals like the Hydras, sponges, Clavellina, many worms and worm-like animals, as well as the early stages of the embryo of Metazoa. We find no difference here between the soma and the germ-plasms, as in each particle of their bodies lie totipotent *I*-cells.

In connection with this we see a *late* (?) separation of the sexual germ (especially in plants). And possibly also in connection with it, comes the absence of sexual dimorphism in the vegetative phase of life of these organisms.

(b) The other pole is occupied by such organisms as *Ascaris*, many insects, and others, whose sexual germ begins its clearly defined and individuated course almost immediately after the first dividing of the fertilized egg. These animals are totally indivisible; they are strictly individual.

(c) The greater portion of multicellular animals occupy an intermediate position between these two poles. Up to a certain moment of their lives they are decidedly "divisible" (experiments with the blastomeres of the Sea-urchin); then, with the limitation of the potential possibilities, there begins a crystallization of the "individuality"—a process which sometimes coincides with the visible individualization of the sexual-germ, and sometimes with a definite aspect of the developing germ.

9. *About Death and Immortality.* Weismann's thesis, that death appears when the soma individuates, retains its correctness. But the immortal germ-plasm is located not only in the differentiated sexual cells, but in all totipotent cells in general, i.e. also in *I*-cells. Therefore all organisms with *I*-cells—plants, Hydras, sponges and so on—are potentially just as immortal, as the germ-plasm. Death comes not the moment the organism passes into a multicellular state; but when the sexual cells turn off along their own special germinating course.

Taking the final result, we find that death is connected with the installing of organic individuality. All "divisible" organisms are potentially immortal. All "indivisible" ones are mortal;—only their germinal plasm is immortal.

10. *About Somatic Mutations.* Once there is no decided difference in plants and similar organisms, between the "soma" and the germinal plasm, the hereditary alterations cannot remain confined to the region of sexual cells alone. Every part of the plant "soma" in which there are totipotent cambium *I*-cells, can lay the beginning of a "somatic" or

vegetative mutation. Such is probably the origin of all somatic and bud variations and mutations.

These changes can sometimes be strictly hereditary, and obey entirely the action of Mendel's law of segregation (Emerson, 1922). There is consequently no occasion whatever for not admitting the "somatic" mutation as a *true* one, as the process of change took place in the germinal plasm.

In connexion with vegetative mutations is the capacity for vegetative segregation. But this phenomenon has been as yet established only for plants and hydras.

Why then can many vegetative mutations not be passed on through sexual propagation? Probably because these mutations, having touched only the plasm of the *I*-cells, and not the very nucleus, were unable to penetrate into the whole genotypical mass of the characters of the given organism. These changes, like all such lasting modifications (Tollas, 1913), are passed on during the sexual act. After the synaptic phenomena and reduction processes, they generally disappear.

(May we possibly look here, in this category of phenomena, for the key to the understanding of the so-called "plasmatic" heredity?)

The appearance of "true," that is, factorial mutations, is usually connected with a definite perceptible period, with the process of the maturing of the sexual cells—strictly speaking with the synaptic stage—which does not occur while the usual *I*-cells become active.

The question of the way in which vegetative mutations (= lasting modifications) can turn from being non-hereditary into hereditary, is still absolutely open.

It is possible, that when vegetative mutations which obey the action of Mendel's law were obtained, processes analogous to the synaptic ones did take place in the *I*-cells. This is one of the special subjects for cytological investigations.

On finishing this study, I am bound to say the whole question of vegetative heredity presents a vast and hardly cultivated field for every kind of research. In my Essay I have made an attempt to throw a little light upon this intricate and dark region.

PETROGRAD,  
May 10th, 1923.

P.S. While finishing writing this Essay I received from Germany, from Mr W. Goetsch, a series of reprints, among which I found one paper

the contents of which were of exceeding importance for me. (Unfortunately, neither the name of the magazine, nor the place of publishing, nor the date is mentioned<sup>1</sup>.) From the few lines of this paper it is clear that Mr Goetsch has also succeeded in the synthesis of those organisms which I have called oligactoids; he obtained them by serial grafting of Hydras—*Pelmatohydra oligactis* and *Hydra attenuata*.

In this way my facts have found full confirmation in this observer's experiments. There is nothing said about the segregation of oligactoids in that extract.

<sup>1</sup> Now issued in *Zool. Anzeiger*, Vol. LVI. 1923.

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#### EXPLANATION OF PLATES IX—XVII.

- Fig. 1. Budding of a sectorial chimaera. *B.Ch.* chimerical bud. Exp. 118, 31. viii. 21.  
 Fig. 2. Budding of a sectorial chimaera. *B.* vestige of a bud thrown off; *B.Ch<sub>1</sub>* first bud of chimerical constitution; *B.Ch<sub>2</sub>* "cone of growth" of the second chimerical bud. Exp. 168, 8. vii. 22.  
 Fig. 3. The same bud (*B.Ch<sub>2</sub>*) on 10. vii. from the side.  
 Fig. 4. The same from above.



Fig. 5. Unequal distribution of body substances on a chimerical bud. Exp. 118 B, 31. viii. 21.

Fig. 6. An artificial sectorial chimaera. Exp. 392, 14. vii. 23.

Fig. 7. Hypostome of an artificial sectorial chimaera from the side. Exp. 121, 4. ix. 21.

Figs. 8-14. The fate of a sectorial chimaera. Exp. 105, 20. viii. 21.

Fig. 8. A day after operation. Independent contraction of tentacles.

Fig. 9. The beginning of spreading of the brown substance. 24. viii.

Fig. 10. The brown substance has reached the head and the stalk. 26. viii.

Fig. 11. The beginning of fusion of two heads.

Fig. 12. A bud-rudiment appearing in budding zone (seen from above). Eight tentacles appearing simultaneously.

Fig. 13. Regulation of the hypostome, from the side. 7. ix.

Fig. 14. The same from above.

Figs. 15-19. Insertion Exp. 164, 28. vi. 22.

Fig. 15. A day after operation. Red head has passed through the body wall of the outer brown hydra.

Fig. 16. Same, from the top. 30. vi.

Fig. 17. Brown hydra becoming free from the inner red component tears the latter in half. 2. vi.

Fig. 18. Same, from the back.

Fig. 19. Chimerical preparation after eating some *Daphnias*. 3. vii.

Figs. 20-37. Insertion Exp. 168, 29. vi. 22.

Fig. 20. The day of operation.

Fig. 21. The following day; rupture of the inner brown component in two and crawling out of its halves through the apertures of the red hydra. 30. vi.

Fig. 22. Red hypostome organizes a "field" and builds up from the brown matter a tentacle. 3. vii.

Fig. 23. Two tentacles added.

Fig. 24. Forming of a new chimerical head *B*. *A*. head of fundamental brown hydra. *B* 1 vestige of a thrown off bud of type *oligactis*; *B* 2 chimerical bud; ♂ testes. 7. vii. (Cf. Fig. 2.)

Fig. 25. Chimerical head *B*, magnified.

Fig. 26. The same from the other side.

Fig. 27. Abdominal part of the fundamental brown hydra. *A*. begins to grow. *B* 2 first chimerical bud; *B* 3 second chimerical bud. 10. vii. (Cf. Figs. 2-7.)

Fig. 28. Regulation of the tentacles on chimerical head; magnified.

Fig. 29. A case of abnormal regulation of one of the tentacles. Autotransplantation of the upper part upon the base of the same tentacle.

Fig. 30. Further stage.

Fig. 31. Head end of the same, magnified.

Fig. 32. Regulation of the abdominal part of the fundamental brown hydra. Heteromorphic budding zone in its middle with eight buds (*B*' 1-*B*' 8). 17. vii.

Fig. 33. Head end of the same, magnified. Regulation of red tentacles.

Fig. 34. Depression of preparation. 21. vii.

Fig. 35. Beginning of new budding and growth of connecting channel. 24. vii.

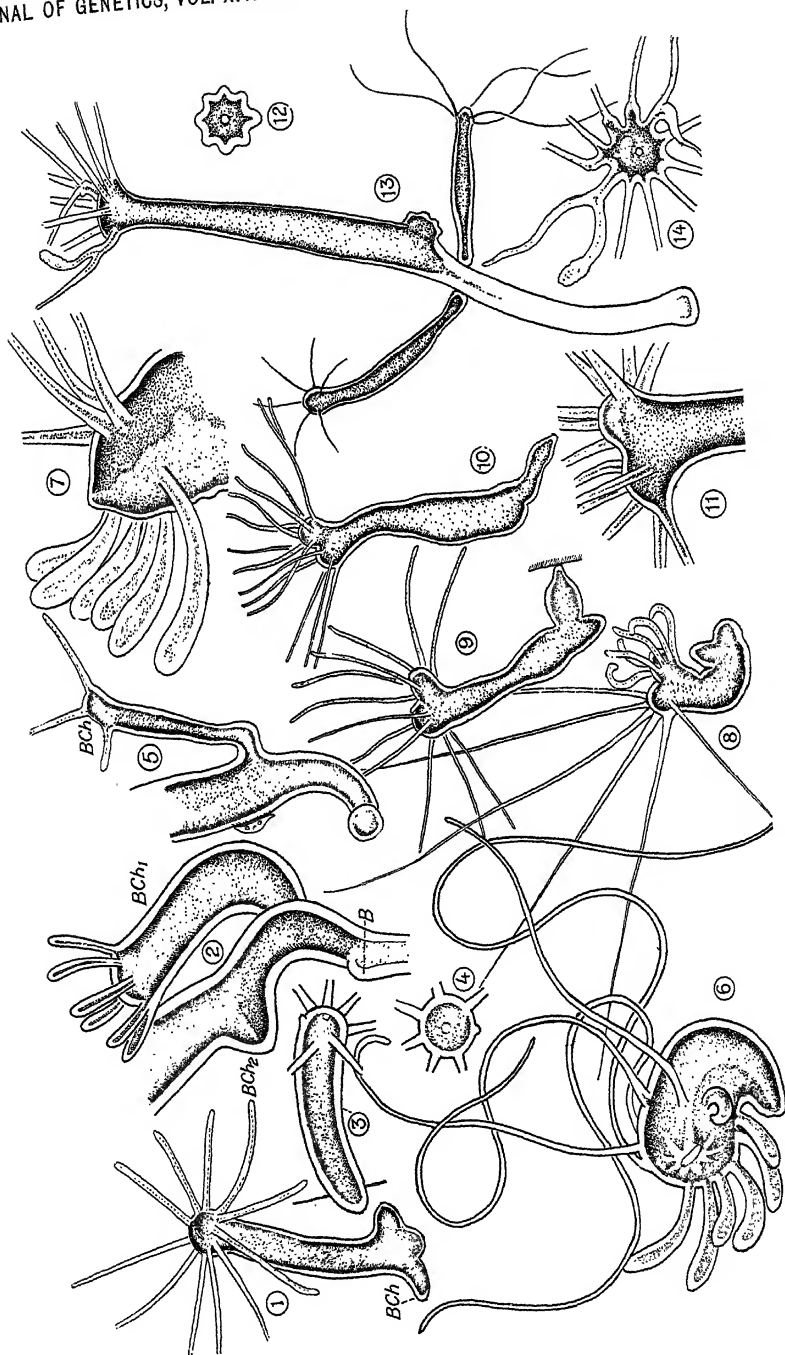
Fig. 36. Segregation of the regulated hydra *B*: buds 5 and 6—*oligactoids* (*Ph.*); *B* 7, *oligactis* (*P.*); *B* 7 and 8, buds of indefinite character. 28. vii.

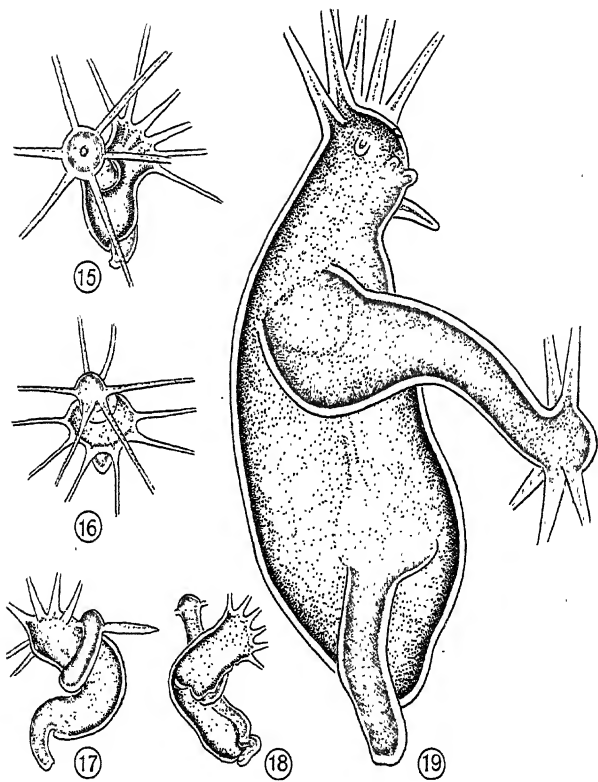
Fig. 37. Same preparation on 2. viii.

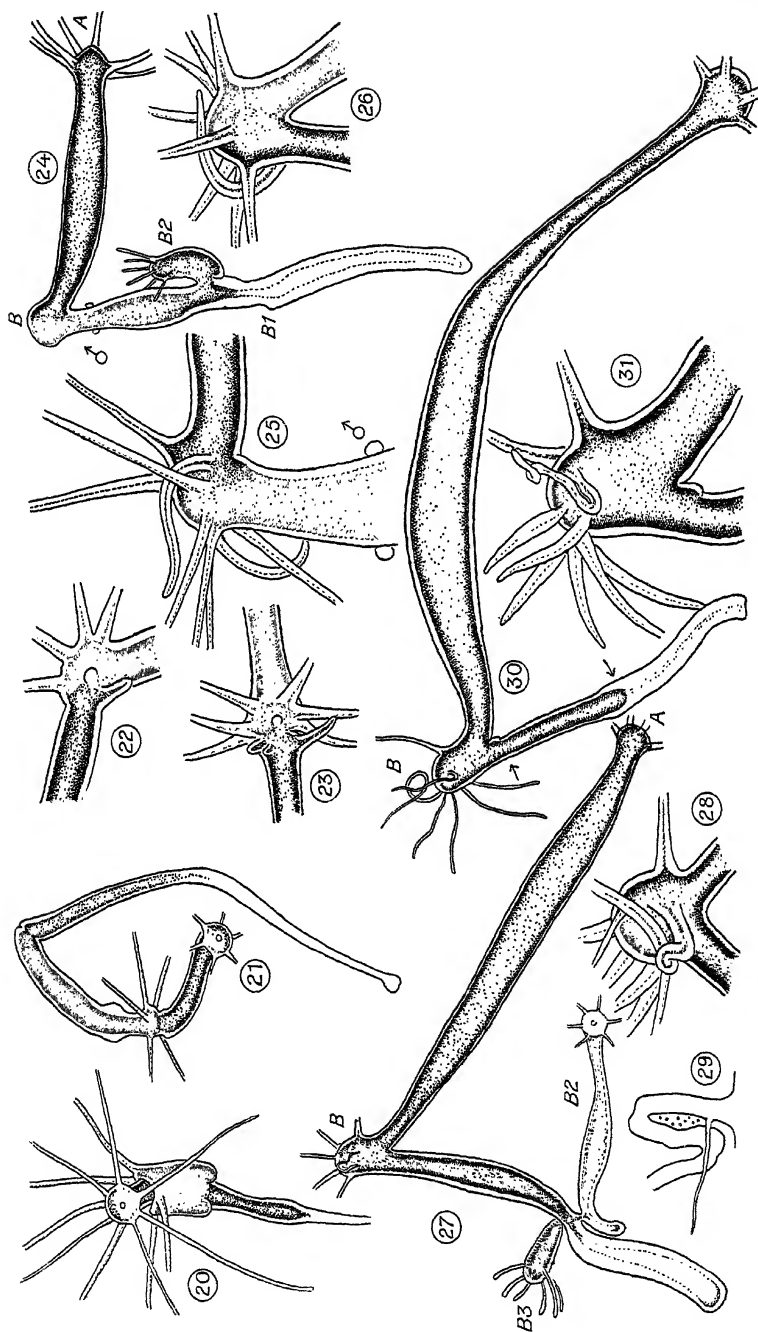
Figs. 38-46. Exp. of Insertion 178, 30. vi. 22.

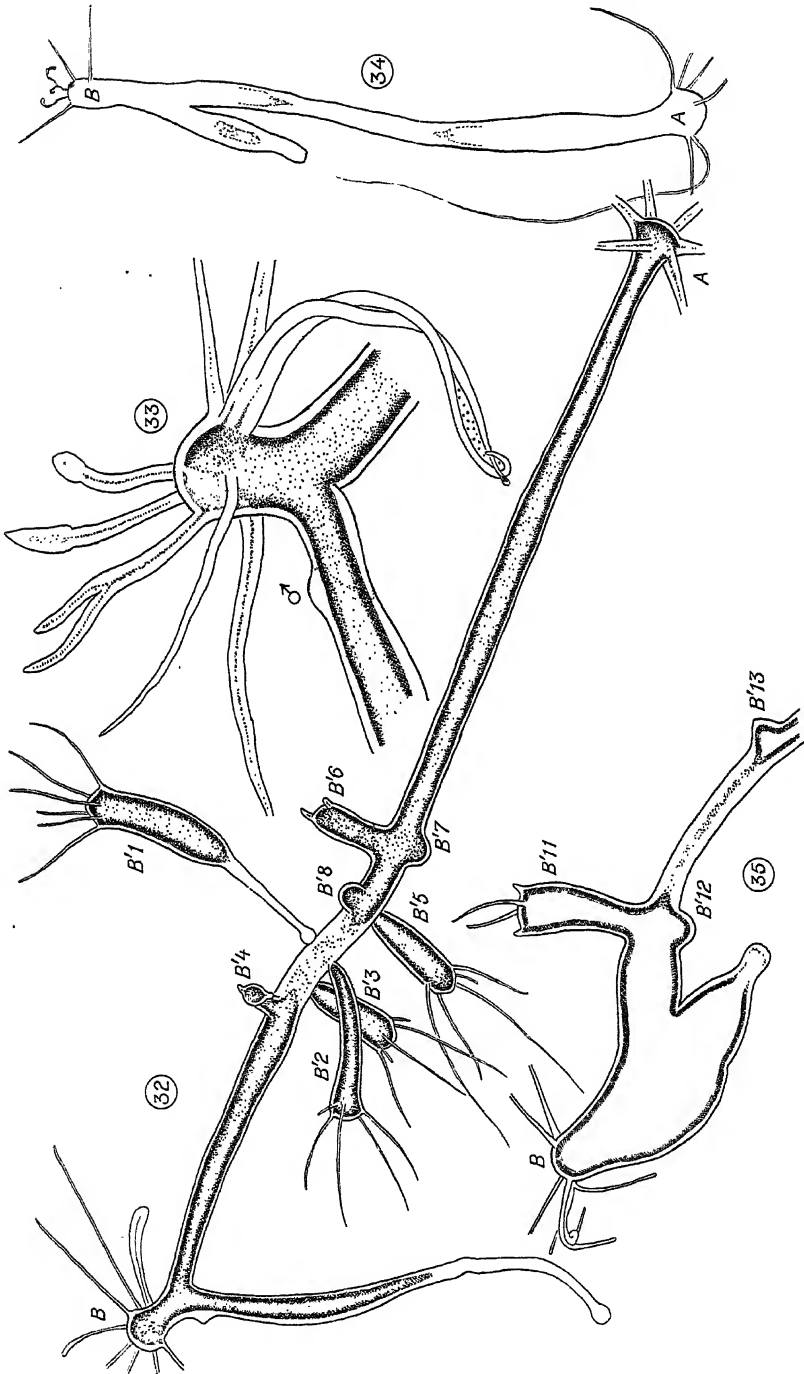
Fig. 38. Insertion, seen from above.

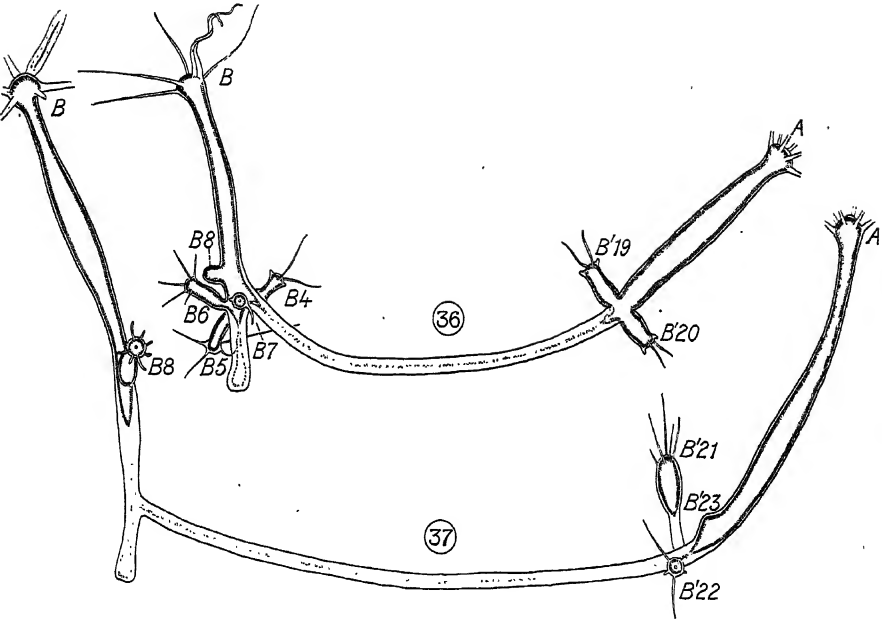
Fig. 39. Beginning of liberation on the part of the inner hydra. 2. vii.

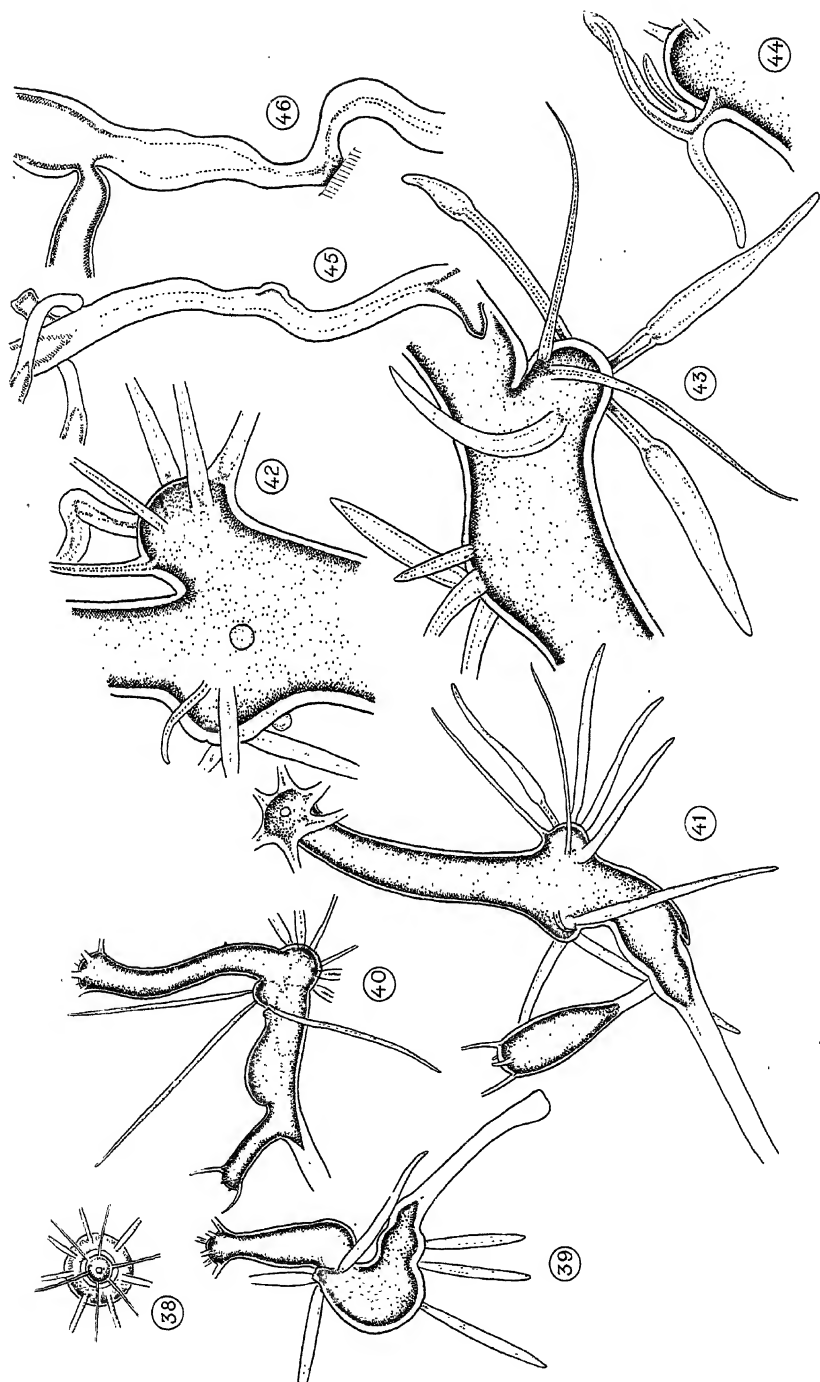


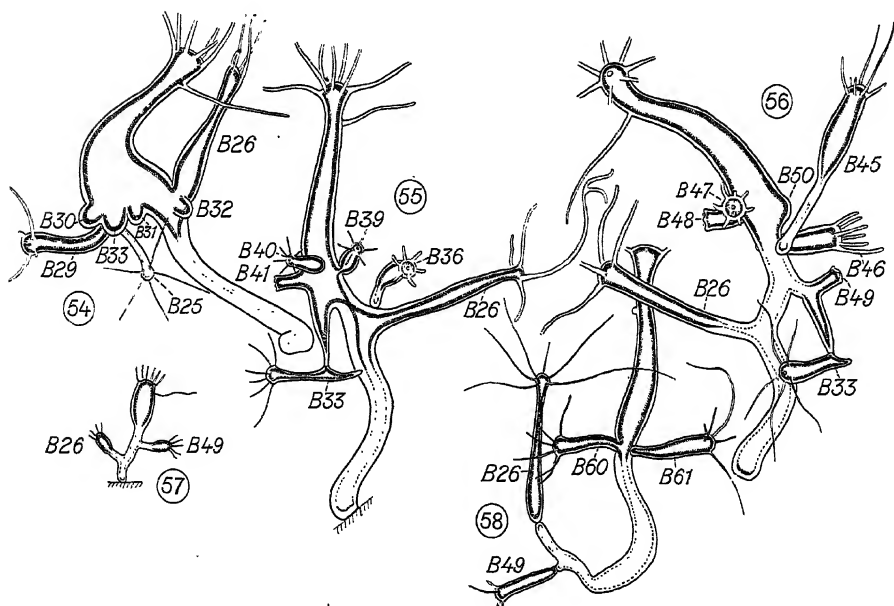
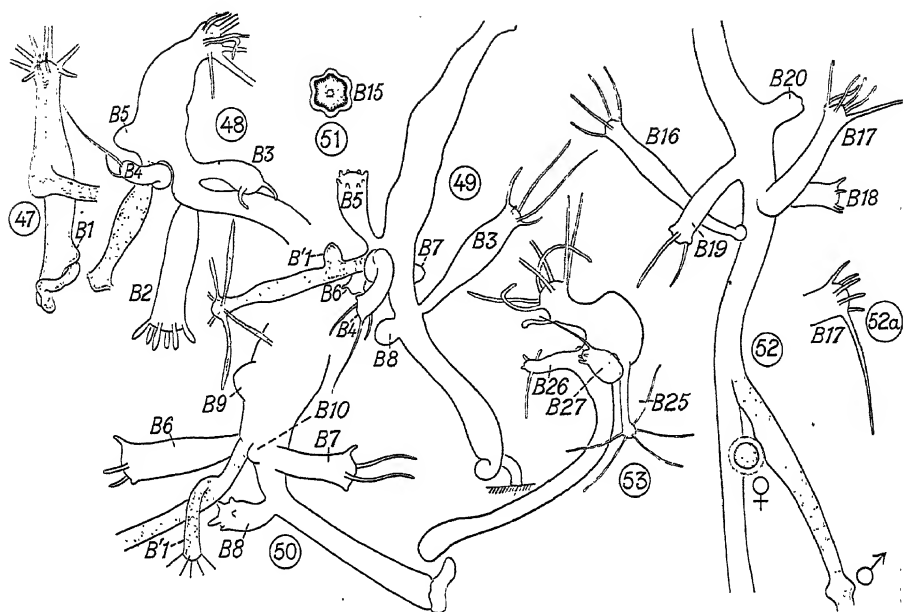




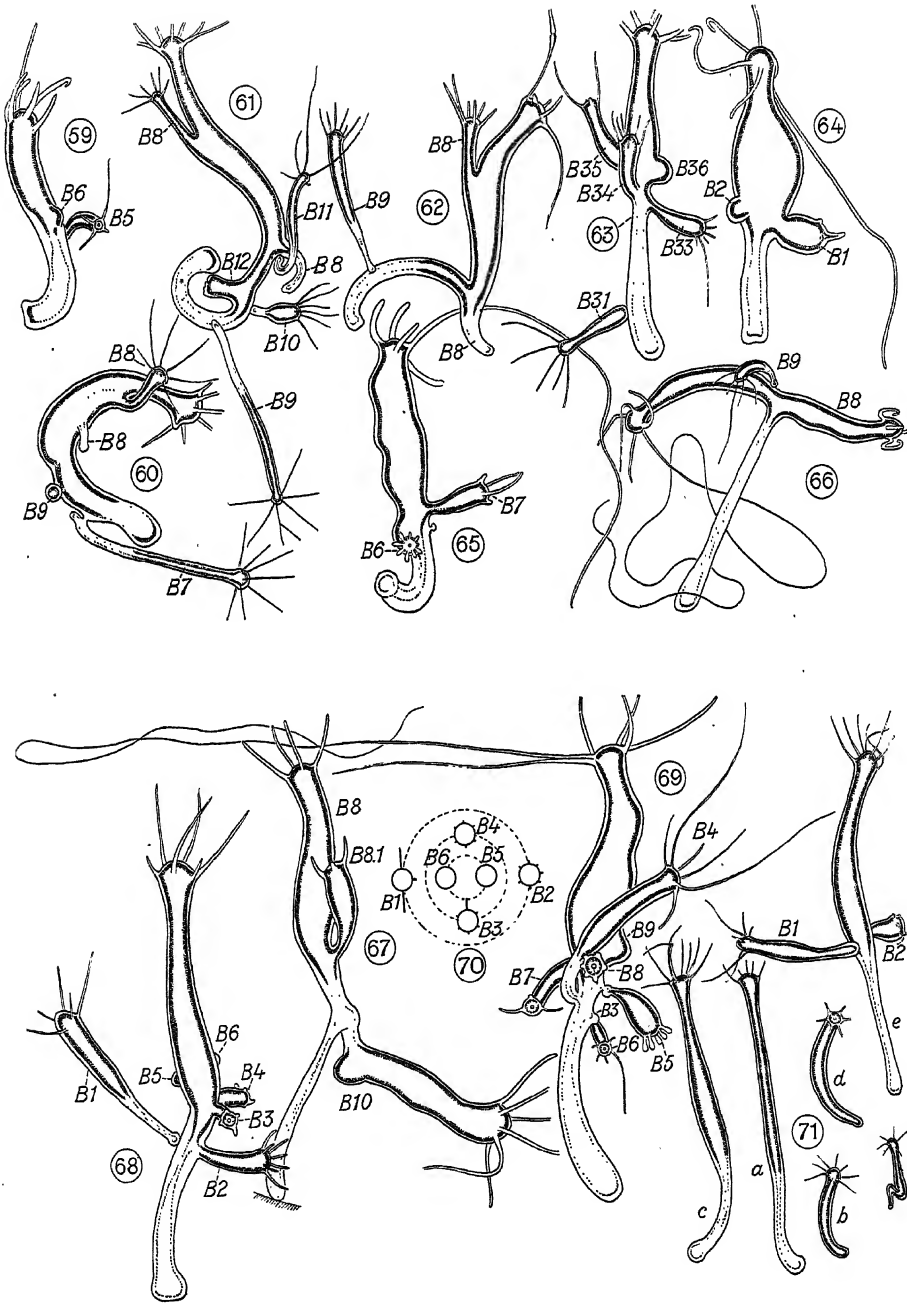


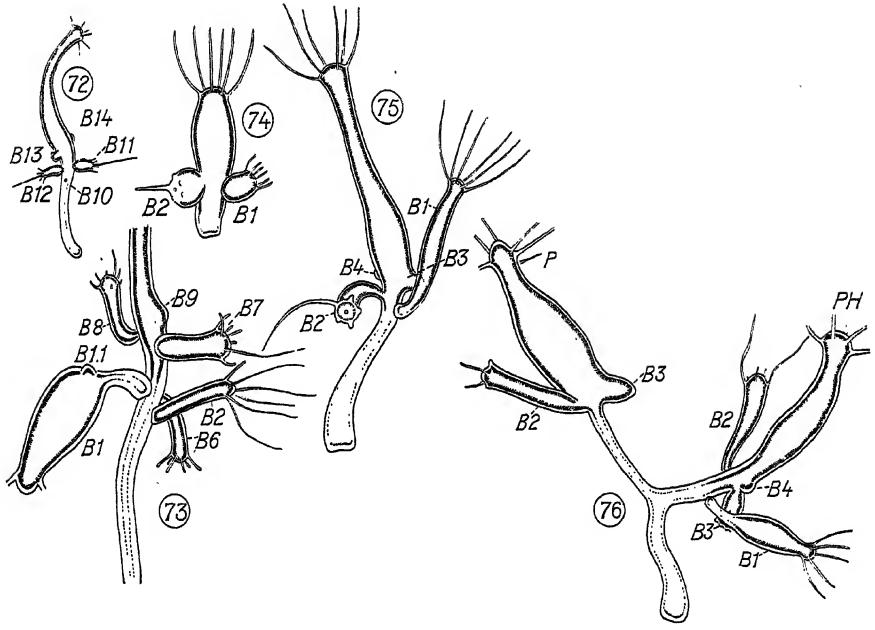














- Fig. 40. Dividing of the hypostome into two heads.  
 Fig. 41. The differentiation of two new heads. 5. vii.  
 Fig. 42. The same, enlarged. One head with three red tentacles, two brown ones, and one pushed out by rudiment of a new brown tentacle. The other head with three red and one brown tentacles; ♂ testes.  
 Fig. 43. Regulation of new head: three red tentacles lifted up by rudiments of three brown tentacles. 7. vii.  
 Fig. 44. Another instance of lifting up of a red tentacle.  
 Fig. 45. Appearance of glandular cells in the "stalky" zone. 24. vii.  
 Fig. 46. The preparation fixed by its sole. 30. vii.

Figs. 47-58. Segregation of *P.Ch.* from Exp. 167.

Dotted part (*H.*), separating red *vulgaris* component; *P. oligactis*; *Ph. oligactoids*; *Ph\**. *oligactoids* with a particularly long tentacle.

- Fig. 47. 5. vii. *B* 1, first bud.  
 Fig. 48. 8. vii. *B* 2, *Ph.*; *B* 3, *P.*; *B* 4, *Ph\**.; *B* 5, bud rudiment; *H.* red component above the budding zone.  
 Fig. 49. 9. vii. *B* 3, *P.*; *B* 4, *Ph\**.; *B* 5, *Ph.*; *B* 6, *P.*; *B* 7, *B* 8, bud rudiments; *H.* red component in the middle of budding zone; *B'* 1, its own bud.  
 Fig. 50. 10. vii. *B* 6, *P.*; *B* 7, *P.*; *B* 8, *Ph.*; *B* 9, *B* 10, bud rudiments; *H.* and *B'* 1 as above.  
 Fig. 51. Appearance of tentacles on the bud of type *Ph.*; 6 tentacles simultaneously.  
 Fig. 52. 17. vii. *B* 16, *P.*; *B* 17, *Ph\**.; *B* 18, *Ph.*; *B* 19, *P.*; *B* 20, *Ph.*; *H.* red component below the budding zone. It possesses two testes (♂) and one fertilized egg (♀).  
 Fig. 53. 27. vii. *B* 25, *Ph.*; *B* 26, ?; *B* 27, *Ph\**.  
 Fig. 54. 29. vii. *B* 25, *Ph.*; *B* 26, ?; *B* 29, *P.*; *B* 30-33, bud rudiments.  
 Fig. 55. 2. viii. *B* 26, ? rests on the stalk; *B* 33, ? on a "pedestal"; *B* 36, *Ph.*, bud on the base of *B* 26; *B* 39, 40 and 41, *Ph.*  
 Fig. 56. 6. viii. *B* 26, the same; *B* 33, the same, "pedestal" develops a bud *B* 49, *Ph.*; *B* 45, *P.*; *B* 46, 47, 48, *Ph.*; *B* 50, 51, bud rudiments.  
 Fig. 57. 12. viii. Preparation in the state of a "colony." *B* 26 and *B* 29 rest on the "stolon" —i.e. stalk.  
 Fig. 58. 23. viii. *B* 26 still resting on the base of the stalk; *B* 49, *id.*; *B* 60, 61, *Ph.*

Figs. 59-72. Segregation of oligactoids in Exp. 164.

- Fig. 59. Segregation of line *B* 2. Buds of the second generation *G*<sub>2</sub>, *B* 2.5 and *B* 2.6. 15. vii.  
 Figs. 60-62. Line *B* 2. Natural complantation between the maternal hydra and its bud (*B* 8). *B* 7, *Ph.*; *B* 9, rudiment of the bud. 22. vii.  
 Fig. 61. 27. vii. *B* 8, head of the bud *B* 8; *f* 8 its foot, organizing a new budding zone with a bud *B* 11, *P.*; *B* 9, *Ph.*; *B* 10, ?; *B* 12, *Ph.*; buds on fundamental budding zone.  
 Fig. 62. 29. vii. The stalk of *B* 8 (*f* 8) supporting the whole preparation; *B* 9 rests on the fundamental stalk.  
 Fig. 63. Line *B* 17, type *Ph\**. Active moment of vegetative segregation. *B* 31, *Ph.*; *B* 33, *Ph\**.; *B* 34, *Ph.*; *B* 35, *P.*; *B* 36, ?.  
 Figs. 64-67. Oligactoid of Line *B* 2.2 (type *Ph\**.). Beginning of segregation. *B* 1, *Ph\**.; *B* 2, ? 16. vii.  
 Fig. 65. 20. vii. *B* 6, *Ph.* with 9 tentacles; *B* 7, true *P.*  
 Fig. 66. 3. viii. Depression. *B* 8 rests on the budding zone. *B* 9, *Ph\**.  
 Fig. 67. 6. viii. "Longitudinal division," parting asunder of parental hydra and its *B* 8: *B* 8.1, a bud of *G*<sub>1</sub>; *B* 10, rudiment of the bud of *G*<sub>2</sub> (*Ph.*).  
 Fig. 68. Line *B* 2.5. 20. vii. Segregation (*G*<sub>3</sub>): *B* 1, *P.*; *B* 2, *Ph.*; *B* 3, ?; *B* 4, *Ph\**.; *B* 5, *P.*; *B* 6, ?.  
 Fig. 69. Line 4.1. 23. vii. Segregation of the oligactoid (type *Ph\**.); *B* 4, *P.*; *B* 5, *Ph.*; *B* 6, *Ph\**.; *B* 7, *P.*; *B* 8, *Ph.*; *B* 9, ?.  
 Fig. 70. Line 4.2. A scheme of regular alternatively opposite position of buds.

- Fig. 71. Line 4.1. Individuated buds *Ph.* of  $G_3$ : *a*, *B* 9; *b*, *B* 10 (8 tentacles); *c*, *B* 11 (8 tentacles), *d*, *B* 12; *e*, *B* 13, *Ph*\*. with buds of  $G_4$ ; *B* 13.1, *P.* and *B*. 13.2, *Ph.*; *f*, *B* 16.
- Fig. 72. Line 17.1. Segregation giving two oligactoids of the type *Ph*\*, *B* 11, 12; *B* 10, *Ph.*; *B* 13, *Ph.*; *B* 14, ?.
- Figs. 73-75. Exp. 170. Beginning of segregation of regulated chimaera. *B* 1, *Ph.* with *B* 1.1; *B* 2, *Ph.*; *B* 6, *Ph.*; *B* 7, *Ph*\*.; *B* 8, *Ph.*; *B* 9, ?. 30. vii. 22.
- Fig. 74. Line *B* 6. Oligactoid 6.2 with its first buds, *B* 1, *Ph.*; *B* 2, *Ph*\*.; 6 viii. In contracted state.
- Fig. 75. The same oligactoid, expanded. Note the shape of tentacles in *B* 2.
- Fig. 76. Exp. 168. Budding of a "double headed" hydra of  $G_1$ ;  $B_1$  oligactoid *Ph.*, segregating in *B* 1.1, *Ph.*; *B* 1.2, *P.*; *B* 1.3, *Ph.*; *B* 1.4, ?.  $B_2$  oligactis, giving true *P.*-buds: *B* 2.1 and *B* 2.2. 6. viii. 22.

## EXPLANATION OF GENEALOGICAL TABLES.

Table I. Segregation of the parental chimaera (*P.Ch.*) from Exp. 164.

Table II. Segregation of the Oligactoid of Line *B* 2 (Exp. 164).

Table III. Segregation of the Oligactoid of Line *B* 4 (Exp. 164).

Table IV. Segregation of the Oligactoid of Line *B* 17 (Exp. 164).

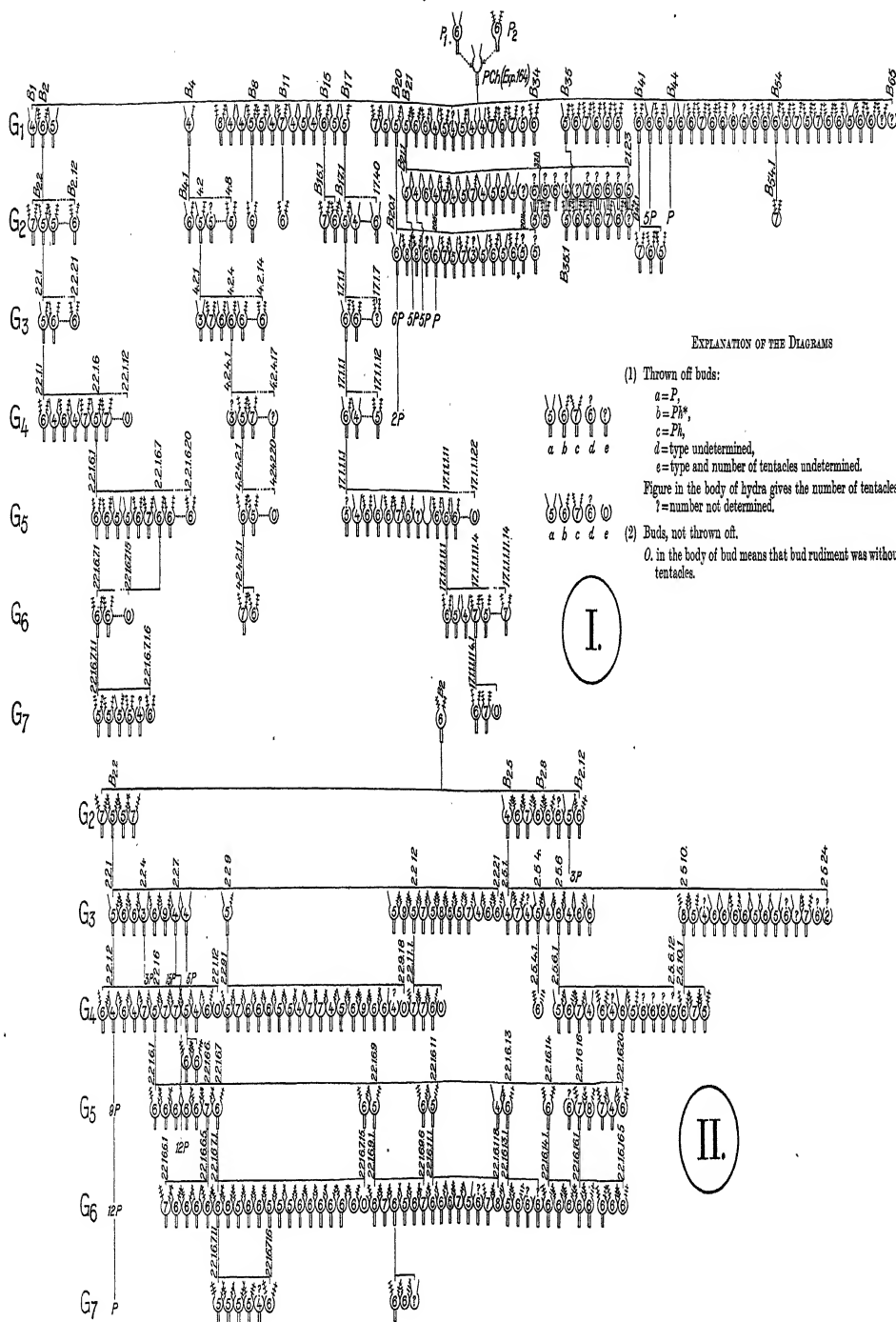
Line *B* 17 in my Preliminary Account (*Biol. Deuts.* XLIII, 1923) is named *Ph.* 8. Slightly altered order in disposition of buds and numbers of tentacles due to the more correct study of records.

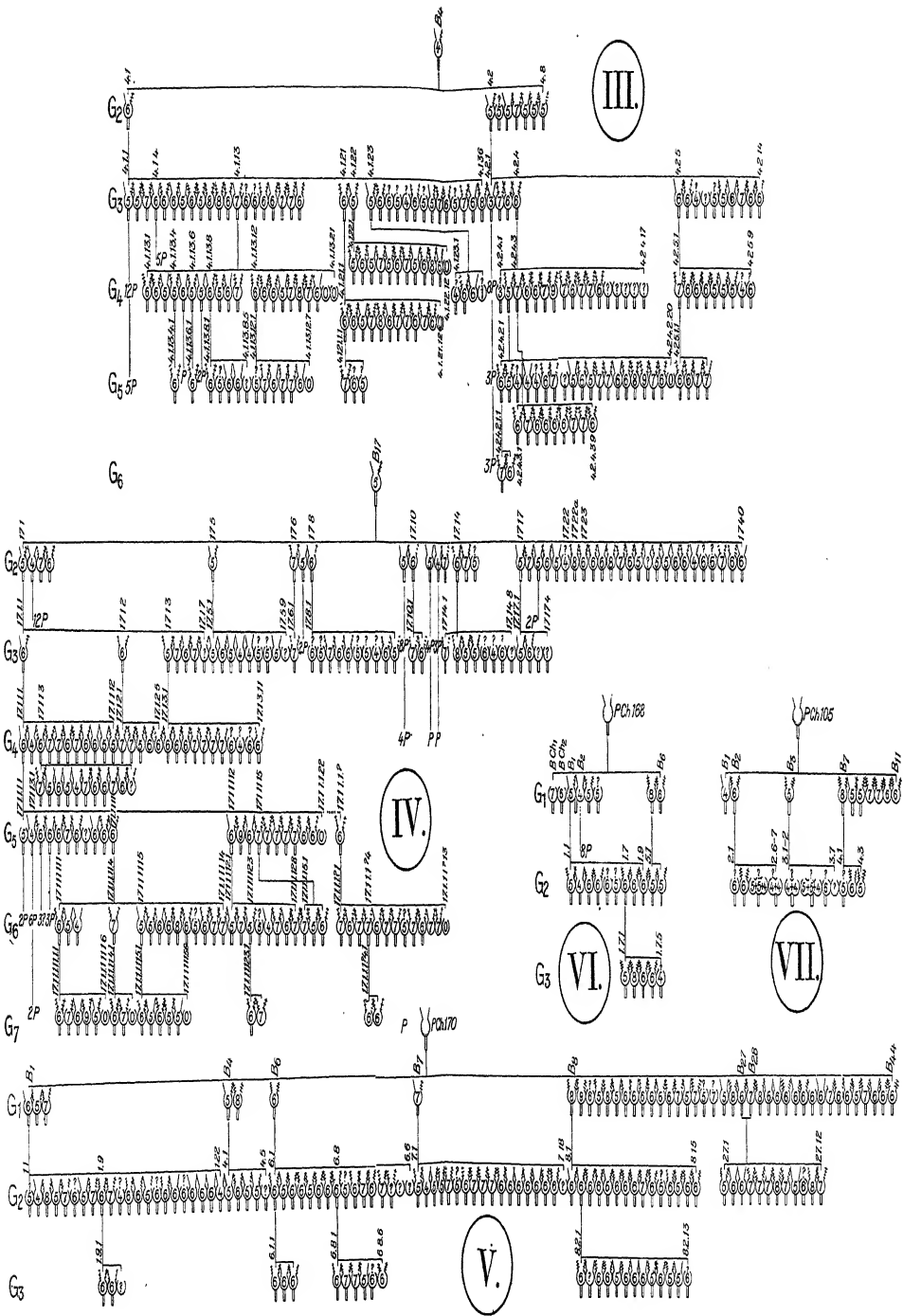
Table V. Segregation of the parental chimaera (*P.Ch.*) from Exp. 170.

Table VI. Segregation of the parental chimaera (*P.Ch.*) from Exp. 168.

Table VII. Segregation of the parental chimaera (*P.Ch.*) from Exp. 105.











# CROSSES WITH SIAMESE CATS

By K. TJEBBES, D.Sc.

(With One Plate.)

## *Introduction.*

MANY years ago Bateson (1909) drew attention to *Siamese Cats*, recommending these peculiar animals as subjects for genetical investigations. Bateson supposed them to represent an analogue to the well-known Himalayan rabbits. From private communication with Mr Bateson I learned that Miss Durham and Mr Backhouse had gathered some evidence about the genetics of Siamese cats, but I have not heard of any further experiments, and, as far as I know, nothing was published.

After having worked on Siamese and other cats for some years I happened to show the short records of my results to Mr Bateson, who encouraged me to publish them, meagre as they are. Their incompleteness is chiefly due to the fact, that Siamese cats are rather exacting animals. They do not like being out of doors, they cannot be kept in pens and will not thrive without the company of man. They need the cosiness and warmth of a human dwelling and must be treated as pet animals. The females are shy and mating is often difficult. Further: the greater part of my cats are the offspring from a cross between white Persian and Siamese. The white Persians are perhaps still more difficult to breed than the Siamese. The weakness of these cats is very striking and their females are utterly bad mothers: they often eat their kittens at birth or starve them to death a few days after, being wearied of their nursing duties.

These difficulties and the high costs of the experiment may account for the small number of individuals (56 cats) upon which the following report is based.

## *Description of Materials.*

The two *Siamese cats* used in my experiments, No. 2 (male) and No. 19 (female), descend both from one pair of animals imported from Bangkok by a Dutch fancier about ten years ago. The strain has always been kept pure and no variability has ever been noticed except slight differences in the intensity of the pigmentation. The dark parts of the fur (face, ears, feet, tail) are from darkest chocolate to almost black;

the lighter parts may vary from a pale greyish buff to a smoky fawn colour. The adult males are considerably darker than either the females or the young animals. At birth the kittens are white or nearly white; after ten or twelve days the pigment begins to develop. The iris of the eyes is blue, but not sky-blue as in blue-eyed white cats; in the Siamese eye there is always a shade of milky grey in the blue of the iris. The deeper parts of the eye are pigmentless.

The *white Persian* female, No. 1, belongs to a strain that during many years was kept by a fancier in my district. It was a very uniform and beautiful strain as far as the long silky fur is considered, but some individuals showed dissimilarity of the eyes, the left eye having a different iris colour (yellow) from the right eye (blue). The female used in my first cross was not affected in this way, both irides being sky-blue. Nearly all the members of this family showed somatic or mental defects (sterility, deafness, dirtiness, inability to withstand the simplest difficulties of a cat's life). This caused much trouble in the course of my experiments. It is to be well understood, that the white Persians, even those with two blue eyes, are not true albinos, but coloured cats, in which pigmentation has been suppressed but for a very slight trace. Most of them have a little black spot—often only a few black hairs—on the neck, the occiput, or the front; and also in the eyes, iris as well as fundus, some pigment is left.

The *tabby* male, No. 16, belongs to the most common type of the ordinary house cat in Holland. The type is in this country called "Cyprian." It is characterized by longitudinal stripes of a yellowish grey on a black coat. On the legs and the tail the stripes run transversely. On the ears and the feet no stripes are visible, but the pigment in those parts is paler than on the back and approaches the colour of the stripes. The pattern is obviously the same as that of so many wild *Felidae*; it is variable in the details, but the dark ground is always preponderant; the stripes are in all cases narrower than the dark parts between them, in a ratio varying from about 40:100 to 60:100. The intensity of the pigmentation is variable too, and two chief types may be distinguished: a dark type (yellowish grey stripes on pure black) and a pale type (pale bluish grey stripes on dark lead grey). The pale behaves as a unifactorial recessive to the dark and is obviously a dilute form, comparable with the "blue" cats in their relation to the "black" ones.

*Eye Pigments.*

As the pigmentation of the eye has been one of the characters taken into consideration in my experiments I will give a short description of those parts of the eye, where pigment is or may be located.

In the *iris* the pigment may be present in the stroma (outer layer); it is always present in the epithelium (at the inside). When both layers are pigmented the iris is yellow, grey, yellowish grey or brownish grey, and the eye is called "duplex." When the epithelium alone is pigmented ("simplex") the colour of the iris is blue. The opalescent blue of the Siamese is probably caused by the presence of cells containing small white corpuscles in the tissues of the stroma.

In the *fundus* of the eye pigment may be found in the retina, or in the choroid, or in both. Besides ordinary pigment the choroid shows in most coloured cats a so-called "tapetum lucidum," a layer of cells containing guanin crystals, that reflect the yellow, blue and green rays of the light. Although this substance is no true pigment it appears and disappears in a way quite conformable with the behaviour of pigments. I will therefore in the following treat it together with these.

*Scheme of Work.*

I crossed a white blue-eyed deaf Persian female, No. 1, with a Siamese male, No. 2, and a Siamese female, No. 19, with a dark tabby male, No. 16. The first and second generations of these crosses were bred and studied. Further one of the  $F_1$  females of the first cross was crossed back with a male Siamese, and so was also an  $F_1$  female of the second cross. Afterwards an  $F_1$  female of the first cross was mated to an  $F_1$  male of the second, and the same female was also crossed with a tabby male.

The results of all these matings, except the last mentioned, are given in the Tables A, B, and C. The cross of No. 5 with a tabby male gave two whites and three tabbies, whose eyes were not investigated. The ophthalmological investigation of the eyes of Nos. 1-43 was carried out by my neighbour and friend Dr W. Smit, to whom I wish to express my thanks for his invaluable help.

*Coat Colours.*

We find that the white colour is dominant. The  $F_1$  of the first cross (Table A) is uniformly white, the  $F_2$  consists of seven whites and three coloured, and the back crosses all give about 50 per cent. whites.

In the  $F_2$  of the first cross the striped pattern appears, though both grandparents are without stripes. It manifests itself combined with the

TABLE A.  
*White Persian* × *Siamese*.  
Odd numbers, females; even numbers, males.

No.	Coat colour	Hair length	Iris	Tapetum lucidum	Retinal pigment	Which eye described
1	White	Long	Blue	None	None; only in the lower half of the fundus some traces of pigment	Both
2	Siamese	Short	Blue, with a faint shade of grey	None; except a trace of green at the upper edge	None	Both
3	White	Short	Yellow	Green, the lower half covered by pigment	Thick pigment in the lower half, none in the upper	Both
4	White	Short	Yellow	As No. 3	As No. 3	Both
5	White	Short	Yellow	Green; absent in the lower half	None	Left eye
6	White	Short	Blue	None	None	Right eye
7	Black	Short	Yellow	As No. 3	As No. 3	Both
8	White	Short	Grey	Invisible, covered by thick pigment	Very thick pigment all over the fundus	Both
9	White	Long	Blue	None	None	Both
10	Black	Short	Grey	Greyish green, slightly shaded by pigment; absent at the lower edge	Traces over the greater part	Both
11	Striped Siamese	Short	Grey	Silvery greyish green, the peripheral parts covered by pigment	Rather thick at the edge; centre free	Both
12	White	Short	Blue, with a faint shade of grey all over, and traces of yellow at the edge	None	None	Both
13	White	Short	Yellow	As No. 10	As No. 10	Both
14	White	Long	Yellow	As No. 10	As No. 10	Both
15	White	Long	Blue	None	None, except several groups of pigment cells in the lower half	Both
7	White	Long	Blue	None	None, except some traces of spread pigment in the lower half	Both
				None	As No. 15	Both

Siamese colours and I therefore think that it has been brought into the cross by the white Persians. Further we see that two of the  $F_2$  animals in this cross are black, which must be caused by a dominant factor. These black cats, when compared with other solid blacks, show a difference in shade, the colour being a very dark chocolate rather than the coal black often seen in ordinary black cats. It is exactly the same black as shown by adult male Siamese in their darkest parts and I therefore feel inclined to think that the factor responsible for this effect is brought into the cross by the Siamese ancestor.

Although the numbers of individuals are small I have endeavoured to compose a working hypothesis as to the factors that act in my experiment. First I suppose a chromogen factor  $A$ . If this is absent no colour pigments can develop. In the Siamese cats  $A$  is replaced by an allelomorph  $a_1$ , recessive to  $A$ , causing a reduction of pigmentation intensity, which results in the typical Siamese colour arrangement, when combined with the factor for chocolate-black  $B$ . The dominant factor causing suppression of all pigment may be designated by  $D$ .

Regarding the tabby pattern it is known from earlier work that it is caused by a series of allelomorphic factors dominant to solid colour, but the facts related below indicate that full chocolate-black ( $AB$ ) is epistatic over tabby. Neglecting details we can represent the striped condition by  $C$  and the non-striped by  $c$ , but I am fairly convinced that the real nature of the striping is not approached by studying the genetics of striped animals in crosses with non-striped. The phenomenon is of a physiological nature and depends probably on quantitative reactions during the development of the skin and its organs. Most probably the "tabby" pattern is inherent in all non-Siamese cats, and it depends partly on hereditary, partly on purely physiological influences, if and to what extent it will manifest itself in the phaenotype. For the purpose of this paper, however, it seems to me that it is practical to comprehend all those hereditary influences under the collective designation of an allelomorphic system  $C/c$ .

As far as I know, the striped Siamese phaenotype has never been described nor represented; I therefore give photographs in Plate I. The stripes are very faint on the back, which looks like the back of a pure Siamese. On the head, the legs and the tail the stripes are very marked. Just as in ordinary tabbies the ears and feet are pale, which constitutes a sharp contrast to the intensely pigmented ears and feet of the Siamese.

The second cross, Siamese  $\times$  tabby, gives a uniform black heterozygote and a clear segregation in the  $F_2$  (Table B). In this cross the factor that suppresses the formation of pigment in the hairs does not

TABLE B.

*Siamese × Dark Tabby.*

Odd numbers, females; even numbers, males.

	No.	Coat colour	Iris	Tapetum lucidum	Retinal pigment
Parents	19	Siamese	Blue, with a faint shade of grey	None, except a trace of green at the upper edge	None
	16	Dark tabby	Yellowish grey	Green; a small segment in the lower part covered by pigment	Thick pigment in the peripheral and lower parts, rest nearly free
First generation No. 21 × No. 18	18	Black	Yellow	Green; upper part yellowish, lower part bluish	Thick pigment in the peripheral parts and below, rest nearly free
	20	Black	Yellow	As No. 18	As No. 18
	21	Black	Yellow	As No. 18	As No. 18
	22	Black	Yellowish grey	Invisible, covered by thick pigment	Very thick all over
	23	Black	Yellowish grey	As No. 22	As No. 22
	24	Black	Yellow	As No. 22	As No. 22
	25	Black	Yellow	Green; upper part yellowish, lower part bluish	Thick pigment in the peripheral parts and in a segment below, rest free
Second generation No. 21 × No. 18	27	Black	Yellow	As No. 25	As No. 25
	26	Siamese	Blue, with faint traces of grey	None, except a trace of green at the upper edge	None
	28	Siamese	As No. 26	None	None
	29	Dark tabby	Yellowish grey	Silvery grey	Thick pigment in the lower part, rest nearly free
	30	Pale tabby	Yellow	Green	Traces of pigment spread all over the fundus
Back cross No. 21 × No. 26	31	Dark tabby	Yellowish grey	Silver green	Spread pigment all over the fundus
	32	Siamese	Blue, with a faint shade of grey	None	Faint traces
	33	Black	Yellow	Invisible, covered by pigment	Thick pigment all over the fundus

act, and no whites are formed. The occurrence of one pale tabby individual points to the presence of a dilution factor, but as there are no "blues" this factor apparently does not act upon the special chocolate-black characteristic for this series. The back cross No. 21 × No. 26 gave a result conforming to the expectation: black, tabby and Siamese.

The crosses represented in Table C revealed nothing unexpected with regard to coat colours. In both crosses an  $F_1$  female from the first cross white Persian × Siamese acted as the mother, and in consequence half of the offspring is white. No. 5 × No. 32 is a back cross  $Aa_1 \times a_1a_1$  so that only one quarter of the kittens possess  $A$ , and in fact only one black individual was produced.

TABLE C.

*Complicated crosses.*

Odd numbers, females; even numbers, males.

	No.	Coat colour	Iris	Tapetum lucidum	Retinal pigment	Which eye described
$F_1$ (white Persian $\times$ Siamese) $\times F_1$ (Siamese $\times$ dark tabby) No. 5 $\times$ No. 18	35	White	Grey	Green	Traces in the peripheral parts	Both
	37	White	Blue	None	None	Both
	36	Dark tabby	Yellowish grey	Green	Thick pigment in the peripheral parts, rest nearly free	Both
	34	Siamese	Brownish grey	Bluish green, partly covered by pigment	Thick pigment in the peripheral parts, rest free	Left
			Blue with a shade of grey	None	Traces in one sector of the lower half part	Right
$F_1$ (white Persian $\times$ Siamese) $\times$ Siamese No. 5 $\times$ No. 32	38	Black	Grey	Invisible, covered by pigment	Thick pigment all over	Both
	40	White	Yellow, more brownish towards the edge	Green	Pigment at the edge, rest free	Both
	41	White	Grey, more brown towards the edge	Green	Thin pigment, spread all over the fundus	Left
			Blue, as No. 39	None	None	Right
	39	Striped Siamese	Blue, shaded grey, with traces of brown, especially at the edge	None	None	Both
	42	Siamese	As No. 39, but without brown	None	None, except some faint traces of pigment, spread all over the fundus	Both
	43	Siamese	As No. 39	None	None	Both

*Hair Length.*

Long-haired cats have reappeared in the  $F_2$  of the first cross, Table A. Six out of ten individuals were short-haired and four were long-haired, though perhaps not all of them as long-haired as the white Persian female No. 1. All the long-haired animals were white. Long-haired cats have not been used in further experiments, though their high percentage and the probable spurious allelomorphism of this character would make an investigation desirable. Matings of the short-haired heterozygotes with other short-haired cats have not given rise to long-haired ones. As my numbers are too small to allow a definite conclusion I can only say that my results agree with those of other experimenters, that the long-haired condition is a recessive character.



*Heredity of Eye Pigments.*

From a genetical point of view the behaviour of the eye pigments is different according to their localisation. An undeniable relation exists between the mode of inheritance of the eye pigments and those of the hairs. In order to facilitate the discussion I have composed Table D, where all cats whose eyes have been investigated are put together, arranged in groups of coat phaenotypes and divided into columns according to the pigmentation of iris, choroid and retina.

TABLE D.

*Cats arranged in Groups of Phaenotypes.*

Iris:	Yellow				Yellowish Grey				Grey				Blue			
	Present		Absent		Present		Absent		Present		Absent		Traces present	Absent		
Tapetum:	Full	Partial	Spread	None	Full	Partial	Spread	None	Full	Partial	Spread	None	None	Traces	None	
Retinal pigment:	Full	Partial	Spread	None	Full	Partial	Spread	None	Full	Partial	Spread	None	None	Traces	None	
White cats	—	3	—	5 L	—	—	—	—	—	—	9	—	—	1	5 R	
	—	4	—	—	—	—	—	—	—	—	35	—	—	14	8	
	—	6	—	—	—	—	—	—	—	—	41 L	—	—	15	37	
	—	12	—	—	—	—	—	—	—	—	—	—	—	17	41 R	
	—	13	—	—	—	—	—	—	—	—	—	—	—	—	—	
Siamese	—	40	—	—	—	—	—	—	—	—	—	—	—	—	—	
	—	—	—	—	—	34 L	—	—	—	—	—	—	—	19	32	2
	—	—	—	—	—	—	—	—	—	—	—	—	—	26	34 R	28
Striped Siamese	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Black cats	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	24	18	—	—	22	—	—	—	7	10	—	—	—	—	—	
	33	20	—	—	23	—	—	—	—	—	—	—	—	—	—	
	—	21	—	—	—	—	—	—	38	—	—	—	—	—	—	
Dark tabbies	—	25	—	—	—	—	—	—	—	—	—	—	—	—	—	
	—	27	—	—	—	—	—	—	—	—	—	—	—	—	—	
	—	—	—	—	—	16	31	—	—	—	—	—	—	—	—	
	—	—	—	—	—	29	—	—	—	—	—	—	—	—	—	
Pale tabby	—	—	—	—	—	36	—	—	—	—	—	—	—	—	—	
	—	—	30	—	—	—	—	—	—	—	—	—	—	—	—	

The *retinal* pigment is inherited in about the same way as the coat pigments: homozygous white cats and all Siamese have a nearly or totally pigmentless fundus, all black and tabby cats possess smaller or larger quantities of retinal pigment.

The *iris epithelial* pigment, though present even in otherwise non-pigmented forms, is probably thicker in coloured cats. I have not been able to make this certain by anatomical investigation of the iris, but an ophthalmoscopical inspection of the eye is sufficient to show that the quantity of pigment is increased.

The *iris stroma* pigment, on the other hand, though always absent or nearly absent in Siamese, is often found in white cats, both in its yellow and in its pure grey form. The yellowish or brownish grey iris, so common in tabby cats, was in my experiments never found in whites.

The iris stroma pigment is always associated with the presence of a *tapetum lucidum* in the choroid, which is not very strange, as the iris stroma and the choroid phylogenetically have the same origin, both being the continuation of the pia mater.

From the table it is evident, that full retinal pigmentation (so thick that the choroid is made invisible) is found in some of the blacks only. As there are no statistical data about the occurrence of retinal pigment in other black cats I am unable to say whether these heavily pigmented eyes are common in ordinary cats or not. I myself had never before seen a black cat whose eyes did not reflect green rays when meeting a light in the dark. My Nos. 7, 24, 33 and 38 do not reflect a trace of light, nor is there anything to be seen with the ophthalmoscope but a velvety black fundus.

Smaller quantities of retinal pigment, located in the lower part or in the periphery of the fundus, are present in other black cats, in tabbies and in white cats with yellow iris. The white cats with grey iris are poor in retinal pigment, in their eyes the pigment cells usually are spread thinly over the whole surface of the retina.

The blue-eyed whites and the Siamese (the striped Siamese included) are devoid of retinal pigment; or they have some feeble traces of it in the lower part of the fundus. Two of the Siamese have also a trace of *tapetum lucidum* at the upper edge, and these two animals are completely without any trace of retinal pigmentation. There is only one exception to the rule that Siamese are practically devoid of retinal pigment; the left eye of No. 34, and I will later on in this paper return to this quite exceptional case.

Throughout the groups yellow, yellowish grey and grey iris no animal is without *tapetum*, whereas among the cats without iris stroma pigment (pure blue-eyed) no *tapetum* occurs. The Siamese, which have blue iris, but also traces of pigment in the iris stroma (the opalescent corpuscles mentioned above), show sometimes a trace of *tapetum*. On very close inspection I also found very faint traces of brown pigment in the iris of a number of Siamese, especially near the inner edge. On these grounds I have formed the opinion, that in the Siamese eye the choroid and iris stroma pigment are not totally absent, but have undergone a reduction comparable to the reduction of the hair pigment in the same animals, by the replacement of the chromogen factor  $A$  by  $a_1$ . If the pigment

developing factor *B* in an *AA* cat produces the choroid pigment (and the guanin crystals) and the iris stroma pigment, it can in an  $a_1a_1$  animal only give existence to a rudimentary tapetum and to milky corpuscles in the stroma. When crossed with an individual that lacks pigment, but possesses *A*, the Siamese, who carry *B*, can thus produce heterozygotes with pigmented eyes. We must suppose, in the case of the cross white Persian  $\times$  Siamese, that the pigment-suppressing factor *D* acts on the hair pigment only and not on the eye pigments.

Regarding the difference in intensity of the eye pigments it seems possible, that, both *B* and *C* being pigment developers, the pigmentation is more intense in the cases where both factors are in action than in those where only one of them prevails. In this way the difference in retinal pigmentation between Nos. 7 and 10 in Table A, between Nos. 22 and 25 in Table B and several others may be accounted for. Even here we are no doubt concerned with a complicated play of quantitative factors, and it would be idle on the base of the incomplete data I can afford, to draw any far-reaching conclusions. So much, however, is certain, that even the iris stroma pigmentation is subject to quantitative variability. There is one striking detail: that all tabbies have a yellowish grey iris and only partial fundus pigmentation. The grey colour of the iris in these cats is more or less mixed up with yellow or brownish elements, especially towards the edge of the iris. The same phenomenon is sometimes seen in striped Siamese, and even in Siamese and whites who have a tabby ancestor.

In order to understand the iris colours we must remember, that the colour we see is the combined effect of different pigments. The iris-epithelial pigment, which gives us the impression of a blue colour when seen through a completely or nearly transparent stroma, looks more or less yellowish or brownish grey as soon as pigment occurs in that tissue. Besides, the epithelial pigment may be of different force; these different shades of the background, combined with the different colour effects of the iris stroma pigment, when present, give rise to quite a series of iris colours, which are very difficult to describe or to classify.

I have not found any variation in the intensity of the tapetum lucidum. The differences in shade it shows are due to the presence of more or less pigment in the retina, through which we see or do not see the choroid. The only variation the tapetum is subject to is one in extent. In cases where the tapetum is reduced, the remaining segment is to be found at the upper edge of the fundus, in sharp contrast to the retinal pigment, whose last remains are always located in the lower parts.

*Heterophthalmic Cats.*

I have already mentioned the dissimilarity of the left and right eyes in the ancestors of the white Persian female, used in my first cross. I have been able to study a sister to my No. 1, where the left eye was yellow and the right eye blue, just as in the heterozygote No. 5. The blue eye was pigmentless in all parts, the yellow eye had a complete tapetum, but only traces of pigment in the fundus, which also is the case in No. 5.

The most striking thing in this matter is, that there is no retinal pigment in the yellow eye, although the other  $F_1$  individuals, whose iris is also yellow, have "thick pigment in the lower half." This fact, however, is the key to the whole question of the heterophthalmy, and provides an explanation of the occurrence of blue eyes in cats who carry one or more pigment factors, the dominant chromogen allelomorph and a pigment suppressor, that has no influence on the eyes. It is plain, that in these white Persians there must be an agent that balances the action of  $D$ , so that its influence is of a quantitative character and varies in extent as well as in intensity. In other words: the area in which the pigment is not suppressed by  $D$ , and which usually contains both eyes and the neck-spot, may be of different extent and, in some cases, leave one eye (wholly or in part), or both, under the influence of  $D$ , just as it may leave a neck-spot, whether large or reduced to zero.

The fact that the iris stroma (with the choroid) and the retina are of such a different origin is a sufficient explanation for the cases where the iris stroma lies within and the retina out of the pigmented area. The presence of  $B$  and the heterozygous condition of  $D$  in the first generation hybrids of the first cross and in the heterophthalmic Siamese No. 34 may account for the rather intensive retinal pigmentation in those cats, as compared to the very poor pigmentation in the white Persians, even when they are yellow-eyed, and in the Siamese.

The incompleteness of my material does not allow me to enter into more details but I hope that sooner or later the investigation of the eye pigment heredity in cats may be taken up by some institution and that the intricate physiological processes causing the quantitative development of those pigments may be cleared up.

## SUMMARY.

Siamese cats have, instead of normal chromogen a weakened chromogen factor, which in cooperation with pigment factors causes the coat and eye colours characteristic for the breed.

The chocolate black of the Siamese reappears in its full intensity in crosses introducing the normal chromogen and is then epistatic over tabby.

The white Persians used in this experiment do not possess the chocolate black, but carry another pigment factor or factors whose allelomorphs cause tabby pattern, that is, striping and pigment suppression on the feet and ears.

The combination of the Siamese colouring and the tabby pattern can give rise to a new type of cats, the striped Siamese.

The absence of colour in white Persians is caused by a dominant factor suppressing pigment nearly totally in the hairs and elsewhere.

This factor seems, however, to be of a quantitative nature and to be balanced by an agent that causes a certain area to be unaffected by this suppressor. This area varies in extent and may include the eyes (one, both or parts) and a spot on the neck or occiput.

Pigmentation of the iris stroma is always accompanied by the presence of a tapetum lucidum in the choroid.

The heterophthalmy found in some white Persians and also in several hybrids is caused by the quantitative reaction of *D* with the agent that limits its sphere of influence.

Both coat and eye pigments are influenced by quantitative physiological processes whose nature has not yet been disclosed.

#### POSTSCRIPT.

After conclusion of the manuscript three kittens were born from No. 5 by No. 42, one white, one Siamese, one striped Siamese. Another litter from No. 41 by the same father consisted of two whites and two Siamese. The percentage of whites is about 50, as expected. The appearance of the striped Siamese is accounted for by the heterozygotism for *C* of the mother, No. 5, whereas No. 41 is probably without *C*.

One of the white kittens of No. 5 had a very large neck-spot, about one inch in diameter.

There was no case of heterophthalmy in these seven animals.

#### EXPLANATION OF PLATE XVIII.

1. Striped Siamese showing the comparatively pale face and the very faint pigmentation on the feet. Tail dark, ears pale.

2. The same cat, showing longitudinal striping on the neck, transversal striping on the legs and tail. The back is nearly without stripes. Nos. 1 and 2 taken under an anaesthetic in the laboratory of Professor Laqueur, to whom I wish to express my thanks for his help.

3. The same, to show markings on the face.



1



2





## COLOUR INHERITANCE IN SHEEP.

### I. BLACK COLOUR AND BADGER-FACE PATTERN IN WELSH MOUNTAIN SHEEP.

By J. A. FRASER ROBERTS.

*From the Department of Agriculture, University College of North Wales,  
Bangor, and the Animal Breeding Research Department, University  
of Edinburgh.*

(With Two Plates.)

BLACK WELSH MOUNTAIN sheep (Pl. XIX, fig. 4) have been bred pure for a considerable time and are recognised as a breed; it would appear that the flocks now in existence have been built up by the selection of black individuals from ordinary Welsh flocks and that no crossing has been practised outside the Welsh Mountain breed. The coat of these sheep is of a deep uniform black, white fibres having been largely eliminated, and the horns are black. A small amount of white patterning does, however, sometimes occur, the area affected being the tip of the tail.

The peculiar type of pattern to which the name "badger-face" has been given is shown in Plate XX. Essentially it consists in the appearance of two black bars between the eyes merging into a blackish area on the top of the head. The inside of the ears is usually black and there is a black patch underneath each eye. The muzzle is black, the pigmented area extending back for some distance along the lower jaw in a characteristic way, and finally tapering off to a narrow stripe which runs down the centre of the throat. The ventral surface of the body, the inner surface of the tail, and the legs, with the exception of curved areas on the outer surface, are also black. The black area spreads out for a short distance over the flanks on each side of the tail.

While this is the typical pattern there is considerable variation both in the direction of extension and of reduction of the pigmented areas. Individuals are found in which the black areas on the face are reduced, the ventral area is grey, and the throat stripe has disappeared. The most extreme cases of reduction found (Pl. XIX, figs. 1-3) showed only the bars between the eyes, a little black on the ears, muzzle lightly pigmented, a black area on the lower jaw, and greyish legs. More heavily pigmented individuals showed a considerable admixture of black in the



fleece on the breast and round the neck, while some were found which were almost black, but still showed by slight differences in shades of grey and black, lines of demarcation especially of the ventral area. Whether it would be possible to obtain badger-face sheep which are indistinguishable from whole blacks is not known; certainly no lambs of that type occurred during the experiments.

Badger-face sheep crop up sporadically in many Welsh Mountain flocks. Breeders do not object to them, so there is no selection against the type, except that a badger-face ram would not be used. In some parts of Wales they have a definite name—"defaid idloes."

Wriedt<sup>(10)</sup> has described a pattern which occurs in just the same way amongst the sheep of Western Norway and assures the writer that it is identical with that found in Welsh sheep. Heller<sup>(7)</sup> describes a lamb showing this type of patterning which was born in a Rambouillet flock, and states that it occurs among the woolless sheep of Barbados and in crosses of this breed with Southdowns. It is also found among Cheviot and Shetland sheep.

It is of interest to note that Welsh sheep are sometimes seen which appear to be "reversed" badger-faces, i.e. the face is black as is the rest of the body with the exception of the large ventral area which is much lighter in colour.

Ordinary white Welsh Mountain sheep have faces and legs which are frequently slightly tan; in the lamb tan patches are usual, these patches being represented in the adult coat by scattered red fibres. (Badger-face sheep also have these tan areas.) The nose is usually mottled or black, and single small black patches on various parts of the body are not uncommon.

#### EXPERIMENTAL BREEDING, 1923-4.

An experimental flock of eighty sheep of the three types described above was collected in the Autumn of 1923 at the Farm of the University College of North Wales.

The white ewes were drawn from the College mountain flock, the black ewes were obtained from a Black Welsh flock, and the badger-faces were bought up more or less individually from a large number of different flocks. It was decided to select, for the first year's breeding, badger-faces which should conform as closely as possible to the type described. Owing, however, to the difficulty experienced in securing a sufficiently large number, it became necessary to include a proportion in which the pigmented areas were reduced. The very heavily pigmented

type was excluded. Of the 33 ewes obtained, 21 conformed fairly closely to the average well-defined type shown in Plate XX; in six cases the pattern was moderately restricted, while six showed extreme reduction—as in Pl. XIX, figs. 1-3. Three rams were used.

1. Badger-Face—the pattern was well defined and very similar to that shown in Pl. XX.
2. Black—a pedigree Black Welsh Mountain ram.
3. White—a pedigree Welsh Mountain ram.

The following matings were made, viz.: badger-face  $\times$  badger-face, badger-face  $\times$  white (reciprocal), black  $\times$  white (reciprocal), badger-face  $\times$  black (reciprocal). The results are shown in the following tables.

TABLE I.

*Badger-face Ram  $\times$  Badger-face Ewes.*

No. of Mother	Sex of Lamb	Phenotype of Lamb	No. of Lamb	No. of Mother	Sex of Lamb	Phenotype of Lamb	No. of Lamb
18	♂	Badger-face	—	24	♀	Badger-face	38 (1924)
19	♀	"	—	27	♂	"	—
20	♀	"	36 (1924)	28	♀	"	—
21	♀	"	—	29	♀	"	22 (1924)
22	♀	"	51 (1924)	30	♀	"	50 (1924)
23	♀	"	24 (1924)	31	♀	"	52 (1924)

TABLE II.

*Badger-face Ram  $\times$  White Ewes.*

No. of Mother	Sex of Lamb	Phenotype of Lamb	No. of Lamb	No. of Mother	Sex of Lamb	Phenotype of Lamb	No. of Lamb
32	♀	White	59 (1924)	41	♂	Badger-face	—
33	♀	"	—	41	♂	"	—
34	♀	"	25 (1924)	42	♀	White	—
35	♀	"	54 (1924)	43	♀	"	—
36	♀	"	55 (1924)	44	♀	"	7 (1924)
37	♀	"	—	45	♀	"	56 (1924)
38	♀	"	53 (1924)	49	♀	"	—
39	♀	"	—	50	♀	"	—
40	♀	"	29 (1924)	51	♀	"	28 (1924)

TABLE III.

*White Ram  $\times$  Badger-face Ewes.*

No. of Mother	Sex of Lamb	Phenotype of Lamb	No. of Lamb	No. of Mother	Sex of Lamb	Phenotype of Lamb	No. of Lamb
6	♀	White	—	10	♀	Badger-face*	23 (1924)
7	♀	Badger-face	10 (1924)	12	♂	"	8 (1924)
8	♀	"	—	12	♀	"	61 (1924)
9	♀	"	26 (1924)				

\* This lamb is very heavily pigmented but is easily distinguishable from a black because of a large saddle-shaped area which is distinctly greyer than the rest of the body and two white areas on the face below the eyes. There is also a certain amount of white on the nose.

TABLE IV.

*Black Ram × White Ewes.*

No. of Mother	Sex of Lamb	Phenotype of Lamb	No. of Lamb	No. of Mother	Sex of Lamb	Phenotype of Lamb	No. of Lamb
59	♀	Black	—	68	♀	Black	—
60	♀	"	2 (1924)	69	♀	"	—
60	♀	"	3 (1924)	70	♀	"	—
61	♀	"	57 (1924)	71	♀	"	35 (1924)
62	♀	"	6 (1924)	72	♀	"	34 (1924)
62	♀	"	—	73	♀	"	—
63	♀	"	58 (1924)	74	♀	"	—
64	♀	"	—	76	♀	"	9 (1924)
65	♀	"	—	76	♀	"	1 (1924)
65	♀	"	—	77	♀	"	—
66	♀	"	—	78	♀	"	—
66	♀	"	—				

TABLE V.

*White Ram × Black Ewes.*

No. of Mother	Sex of Lamb	Phenotype of Lamb	No. of Lamb	No. of Mother	Sex of Lamb	Phenotype of Lamb	No. of Lamb
1	♀	Black	4 (1924)	4	♂	White	—
1	♀	"	5 (1924)	4	♀	"	—
2	♀	"	—	5	♀	Black	31 (1924)
3	♀	"	33 (1924)				

TABLE VI.

*Badger-face Ram × Black Ewes.*

No. of Mother	Sex of Lamb	Phenotype of Lamb	No. of Lamb	No. of Mother	Sex of Lamb	Phenotype of Lamb	No. of Lamb
13	♀	Badger-face	21 (1924)	16	♂	Black	11 (1924)
15	♀	Black	—	17	♀	"	27 (1924)

TABLE VII.

*Black Ram × Badger-face Ewes.*

No. of Mother	Sex of Lamb	Phenotype of Lamb	No. of Lamb	No. of Mother	Sex of Lamb	Phenotype of Lamb	No. of Lamb
52	♂	Black	—	57	♀	Black	32 (1924)
52	♀	White	60 (1924)	58	♂	"	—
53	♀	Black	39 (1924)	79	♂	"	—
54	♀	"	Still-born	80	♀	"	—
55	♀	"	—	81	♀	"	—
56	♀	"	37 (1924)	82	♂	"	Aborted

Segregation was perfect; every lamb fell unmistakably into one of the three classes. The blacks were indistinguishable as regards colour from Black Welsh sheep; the whites showed no more variation than the slight colour differences found in ordinary Welsh Mountain sheep. The badger-faces, with the exception of one heavily pigmented lamb noted in Table III, were, as would be expected, considerably more

uniform than the original ewes. The proportion of those in which there was a reduction of the pigmented areas was small.

A number of black lambs exhibited the white pattern already described, in addition to a small white patch on the top of the head which does not persist in the adult coat.

TABLE VIII.

*Black Lambs which exhibited "White Pattern."*

Ram	Ewe	Sex of Lamb	Nature of Pattern	
Badger-face	15 (black)	♂	White patch on top of head	
Black	55 (badger-face)	♂	"	and white tip to tail
"	58 (badger-face)	♂	White tip to tail	"
"	69 (white)	♂	White patch on top of head	
"	77 (white)	♂	"	"
"	80 (badger-face)	♂	"	and white tip to tail
"	54 (badger-face)	♂	White patch on top of head	
"	68 (white)	♂	"	"
"	82 (badger-face)	♂	"	"

#### DISCUSSION AND INTERPRETATION OF RESULTS.

##### 1. The relation of badger-face pattern to white colour.

There would appear to be strong indications that the badger-face pattern behaves as a simple recessive; the badger-face ram mated to badger-face ewes gave 12 badger-face lambs and the same ram mated to white ewes gave 16 whites and 2 badger-faces. That a few of the white ewes would prove heterozygous for badger-face was expected as two or three badger-face lambs are born every year in the flock from which these animals were drawn. It is more difficult to explain why the white ram mated to badger-face ewes should give 6 badger-faces and one white. The numbers are, however, too small to exclude the possibility that the white ram was simply heterozygous for the badger-face character. This particular ram was seven years old and a noted sire; he was used because there was no record of his having produced anything but white offspring; this year, however, he was the sire of a badger-face lamb from a pedigree ewe<sup>1</sup>.

That this character is recessive is in harmony with the experience of breeders who get badger-face lambs from white parents, sometimes in pedigree flocks, and with the data collected by Wriedt<sup>(10)</sup> regarding the similar pattern in the sheep of Western Norway.

Further breeding work with these sheep will be carried out and in particular the badger-face lambs produced by the white ram will be tested; in this way the nature of the inheritance of the badger-face pattern as contrasted with white should be definitely established.

<sup>1</sup> A son of this ram sired, this year, five badger-face lambs.

## 2. The relation of black to white colour.

Davenport<sup>(2)</sup> from an analysis of Graham Bell's sheep catalogue; Taylor White quoted by Adametz<sup>(1)</sup> and Hagedoorn<sup>(6)</sup>, the two latter dealing with New Zealand Merino and Dutch sheep respectively, describe a black which behaves as a simple recessive. The black of Karakul sheep is dominant, though in this case it is the lamb's coat which is considered, the fleece of the adult being grey or dirty white<sup>(1, 3, 9, 11)</sup>.

The writer is informed that one or two black ewes are often intentionally kept in Blackface flocks and the general experience of shepherds is that such ewes produce about equal numbers of black and white lambs. This is a strong indication of a dominant black. The crossing experiments of Elwes<sup>(5)</sup> indicate that the black of Black Welsh Mountain sheep is dominant to white. It is suggested that in the present experiment the results are explicable on the assumption that black is a simple dominant. The black ram mated to white ewes gave 23 black lambs while the white ram mated to black ewes gave 5 blacks and 2 whites, both the latter being from the same ewe. It is to be expected that a proportion of the individuals in Black Welsh flocks would be heterozygous as white lambs crop up occasionally.

There is, however, one exceptional lamb, No. 60 (1924), Table VII, which is white, the other 12 lambs produced by a cross between the black ram and badger-face ewes being black. The black ram thus sired 35 black lambs and one white one. It is interesting to note that Davenport<sup>(2)</sup> also found an exceptional (white) ram which when mated to heterozygous ewes produced nothing but white lambs, but which, when mated to black ewes, gave 20 whites and 3 blacks. He suggested that the explanation might be a failure or reversal of dominance by which the heterozygous individual exhibits what is normally the recessive characterisation. Further speculation however, is, in this case, unnecessary, because the exceptional white lamb is fortunately a male, so his constitution can be thoroughly tested; his mother, too, will be available for further breeding.

It is not intended to convey the impression that the relation of black to white in Welsh sheep is necessarily always one of a dominant black. There are records of the birth of black lambs from white parents, but this subject requires further investigation in order to exclude the possibility that these are really cases of extreme extension of the badger-face pattern. Breeders sometimes refer loosely even to typical badger-faces as "blacks."

## 3. The relation of black colour to badger-face pattern.

Black would appear to be epistatic or dominant. The exceptional lamb already mentioned points to the relation being one of simple epistasis; on the other hand the production of a badger-face lamb from a black ewe mated to the badger-face ram—No. 21 (1924), Table VI—can only be explained on the hypotheses advanced by assuming that the mother was heterozygous for black and at least heterozygous for badger-face. In this case too further work is required to make the position clear.

## 4. White pattern in black sheep.

The white pattern mentioned as occurring in Black Welsh sheep and in some of the experimental lambs as shown in Table VIII is characteristic of many breeds of coloured sheep. Elwes (4) describes it in Manx sheep and Noble (8) in Piebald crosses. Adametz (1) describes it very fully in the case of the Karakul and in Karakul-Rambouillet crosses. He states that his results can be explained by postulating a single recessive factor for white pattern together with another factor which modifies its action as regards the extent of the pattern. He also states that Rambouillets showed in crosses that they too carry these factors though naturally they cannot be expressed. The present experiment throws no further light on the inheritance of this characteristic; its occurrence is simply recorded.

I wish to express my thanks to Professor R. G. White for the interest he has taken in the work and for constant help and encouragement I am indebted to Mr R. M. Greaves, from whom the Black Welsh sheep were obtained, for much useful information regarding this breed.

## SUMMARY.

1. The Black Welsh Mountain breed of sheep, sheep exhibiting the type of pattern to which the name "badger-face" has been given, and ordinary Welsh Mountain sheep are described from the point of view of their coloration.

2.

TABLE IX.

*Summary of Results of Experimental Breeding.*

	Lambs		
	Badger-face	Black	White
(1) Badger-face ram × badger-face ewes gave	12	—	—
(2) Badger-face ram × white ewes	2	—	16
(3) White ram × badger-face ewes	6	—	1
(4) Black ram × white ewes	—	23	—
(5) White ram × black ewes	—	5	2
(6) Badger-face ram × black ewes	1	3	—
(7) Black ram × badger-face ewes	—	12	1

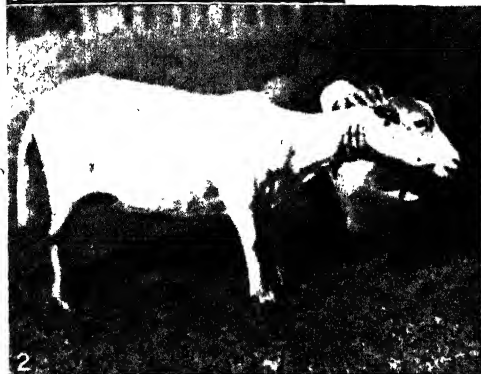
3. It is tentatively suggested that the badger-face pattern is based on a single recessive factor and black colour on a simple dominant (both as contrasted with white) and that the relation of black colour to badger-face pattern may prove to be one of epi- and hypostasis.

4. The case of the exceptional white ram lamb No. 60 (1924) is discussed.

5. The occurrence of "white pattern," viz. a white patch on the top of the head in the lamb's coat and a white tip to the tail, is recorded in the Black Welsh breed and in some of the experimental black lambs.

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Figs. 1-3. Badger-face Ewe showing extreme restriction of pigmentation.  
FIG 4 Black Welsh Mountain Ewe. Figs. 5-6. Badger-face Lambs.







Figs. 1-5. A Typical Badger-face Ewe.



# HYBRIDS OF ♀ *RAPHANUS SATIVUS* L. × ♂ *BRASSICA OLERACEA* L.

By G. D. KARPECHENKO.

(*Plant-breeding Station of the Moscow Agricultural Academy, Russia.*)

(With Two Plates and Three Text-figures.)

CYTOLOGICAL investigations of *Cruciferae*, begun by the author in 1922, proved that *Raphanus sativus* L. and *Brassica oleracea* L. have the same number of chromosomes. As several hybrids of these genera had been described formerly, but not yet cytologically investigated, similar crosses were made by the author and unexpectedly resulted in giving 123 hybrids of *Raphanus sativus* L. × *Brassica oleracea* L. Thus not only the discovery of interesting facts concerning the behaviour of parental chromosomes in the process of formation of sexual cells was made possible, but also the establishment of the morphological peculiarities of the hybrids.

The crosses were made in June of 1922. Altogether 202 flowers of radish (*Raphanus sativus* L. *prol. niger* Pers.) were emasculated and pollinated with pollen from different cabbages: *Brassica oleracea* var. *capitata* L., var. *gongyloides* L., var. *sabauda* L. and var. *gemmifera* D.C. The flowers were isolated in muslin bags, and almost all of the pollinated stigmas set fruit and developed pods with 2 to 4 small shrivelled or normal seeds. These seeds were planted 31 March, 1923, in a box in a greenhouse. The seedlings came up at intervals, taking on an average eight days<sup>1</sup>, but most of the seeds did not germinate at all. On April 10 the seedlings were transplanted into pots and kept in a cooler part of the hothouse, and later on under cold frames. Many plants differed from the radish from the very beginning, having less conspicuously nerved cotyledons, leaves less dissected, and less pubescent or glabrous, and in crosses with red cabbage, an anthocyan colouring of stems and leaves. Although differing in vigour, all hybrids towards the end of May had the appearance of strong cabbage seedlings with 6-7 leaves, and were transplanted into a field-plot at a distance of 1½ metres from each other. Here they began to develop and their differences became more apparent, some hybrids remaining till the autumn small and stunted, others on the contrary reaching an enormous size. Towards the end of July several hybrids were in bloom and about the middle of October out of 123

<sup>1</sup> Whereas seeds of radish and cabbage come up on the 4th day.

## 376 *Hybrids of* ♀ *Raphanus sat. L.* × ♂ *Brassica ol. L.*

hybrids 46 flowered, and 25 plants had flower-buds<sup>1</sup>. Some plants developed pods which did not ripen and gave no seeds.

The hybrids were crossed with their parental forms and with each other, and material was fixed for cytological investigation. In the middle of October of 1923 the plants were minutely studied morphologically and dug up. After the leaves were cut away the roots were stored for planting next spring (1924).

### MORPHOLOGY OF THE HYBRIDS.

Respecting the first hybrid of *Raphanus sativus* L. × *Brassica oleracea* L., made by Sagaret in the middle of the last century, we know that it flowered abundantly but gave only two normal pods, one of which resembled a cabbage pod and the other a radish pod. Seeds of these pods developed weak plants which Sagaret did not study. The second hybrid described by Gravatt in 1914 was of striking vigour. Its branched stem grew out through the ventilator of the roof of the greenhouse and a short way down it on both sides. It had big glabrous leaves resembling those of a cabbage, it blossomed abundantly, bore flowers, but was completely sterile and gave no pods. 15 per cent. of the flowers had eight stamens; the ovaries appeared to be 2-celled like those of the cabbage parent. One slide of the hybrid pistil showed sections of an ovary with three cells. As to the hybrids *Raphanus sativus* × *Brassica oleracea* made lately by Baur we know only that they were very vigorous but completely sterile. This is about all we know concerning the morphology of radish-cabbage hybrids.

For a proper analysis of a hybrid plant a sufficient number of the hybrids and a statement of the characters which most differentiate the two parents are required. We had an unusually large number of hybrids of *Raphanus sativus* L. × *Brassica oleracea* L. and there were no difficulties in establishing the characters necessary for an analysis, as both genera differ sharply in regard to roots, leaves, flowers and fruits, and at the end of the period of vegetation in their general habit also. We begin our description of parental forms and of their hybrids with their habit of growth.

*Habit of growth.* The maternal forms of radish, grown under the same conditions as the hybrids, were in bloom towards autumn, their flower-stalks being 1·7–1·9 metres long, the lower leaves being 30–40 cm. in length, the length of the flowering axis 25–45 cm.

<sup>1</sup> Cabbage flowers in general in its second year of growth whereas radish often bears seeds in its first year, especially if sown early.

The cabbages did not flower and had an ordinary appearance: hearting cabbages had a short stem, the leaves, with exception of a few lower ones, forming the heart; kohlrabi had a thick stem in form of a bulb; Brussels sprouts had a longer stem with big leaf buds in form of small heads; Savoy cabbage had a short stem with crinkled leaves forming a loose bunch. The length of the lower leaves of cabbages was 40–60 cm.; the height of the longest Brussels sprouts did not exceed 70–90 cm.

The radish-cabbage hybrids towards autumn appeared to be strictly polymorphic in their habit of growth and, irrespective of difference in the variety of cabbages participating in the crossing, were generally of three types. The 1st type was a small plant with a very short stem about 30 cm. in length, densely covered, especially at the base, with small leaves; the stem was often branched and bore short flowering shoots 15–17 cm. in length with clustered flowers. The 2nd type of plant consisted of big leafy rosettes of  $1\frac{1}{2}$  metres in diameter, or bushes with short thick branches and big leaves. They flowered sometimes, developing small clusters of flowers.

The 3rd type was represented by plants of a vigorous growth with a strong stem with many branches of  $2-2\frac{1}{2}$  metres in height. They flowered abundantly and their flowering shoots reached a length of  $1\frac{1}{2}$  metres and more. These plants were like wild Cruciferae such as *Sisymbrium* or *Barbarea*, but of a gigantic size. The difference between the hybrids is clearly seen in our photograph (Pl. XXI, fig. 1).

Hybrids of type I are obviously stunted in growth never attaining the size of a flowering radish or cabbage. The stems of cabbages grown beside the hybrids were 1.2–1.5 metres in height. Hybrids of the second

TABLE I.

No.	♀	♂	No. of cross-ings	No. of hybrids obtained	No. of blossoming hybrids 1 year	No. of hybrids with outgrowth on roots	No. of hybrids			Remarks
							I	II	III	
1	<i>Raphanus sativus</i> L. prol. niger Pers.	<i>Brassica oleracea</i> L. var. capitata L.	127	55	35	37	14	29	12	Two intermediate hybrids between the II and III type are included in the II type
2	"	<i>Brassica oleracea</i> L. var. gongyloides L. (Kohlrabi)	32	33	23	21	11	7	15	—
3	"	<i>Br. oleracea</i> L. var. sabauda L. (crinkled leaf cabbage)	27	4	4	1	—	2	2	—
4	"	<i>Br. oleracea</i> L. var. gemmifera D.C. (Brussels sprouts)	16	31	9	16	15	14	2	Two hybrids of an intermediate type between the I and the II are included in the I type
Total			202	123	71	75	40	52	31	

and third type exceed their parent in vigour and were alike in this respect; possibly their difference is due to a slower development of stems in plants of type II.

Transitional forms between these three types occur occasionally as exceptions. This polymorphism of hybrids has been observed for crosses with all varieties of cabbages. Table I shows the respective figures.

There are more rosettes or low "bushes" (type II) in crossings with hearting cabbage; stunted plants occur more often in crossings with Brussels sprouts; developed plants of great vigour are numerous in crosses with kohlrabi. Sometimes in a group of crosses there are only two types. Table II shows, e.g., that the  $F_1$  of the 17<sup>22</sup> crossing was represented by two groups of plants, viz. nine small ones with small leaves and six very full ones with big leaves, but in crosses 23<sup>22</sup> and 4<sup>22</sup> there are all three types of hybrids. The difference between representatives of the three types in one cross was very great. Thus in the cross No. 23, we had plants with a height of 5 cm. only, with small leaves no longer than 26 cm., but we had also in the same cross rosettes with leaves 68 cm. long and plants 250–260 cm. high. This divergence in  $F_1$  was observed in all crosses notwithstanding the small number of hybrids. Their number is too small to establish any definite relations between the three types.

*Stem.* The stem of a flowering cabbage is always glabrous, with a waxy efflorescence, and has a normal pith. The stem of a radish is pubescent or hairy, and hollow; it has often a reddish hue near the nodes and at the base of branches. The hybrids had stems with a normal pith; they were glabrous with a slight waxy efflorescence; almost all plants had a reddish colouring at the nodes, but only 15 plants of the 1st type had pubescent stems. Hybrids of red hearting cabbage were coloured as their parental forms all along their stems. The broadening of stems in kohlrabi is dominant in inter-racial crosses. It is caused by a development of vascular fibres surrounded by parenchyma and connected with the normal peripheral system of fibres only in its upper and lower part of the enlargement (Lund and Kjærskou). Half of the hybrids of radish × kohlrabi developed such bulbs on the stem, but it is interesting that an anatomical investigation of several such "enlargements" in hybrids did not discover any central systems of vascular fibres, and only in one core were there several bunches of fibres, whereas ordinarily they consisted simply of parenchyma, and the vascular fibres were located only along the periphery of the stem. Perhaps it was on

TABLE II.

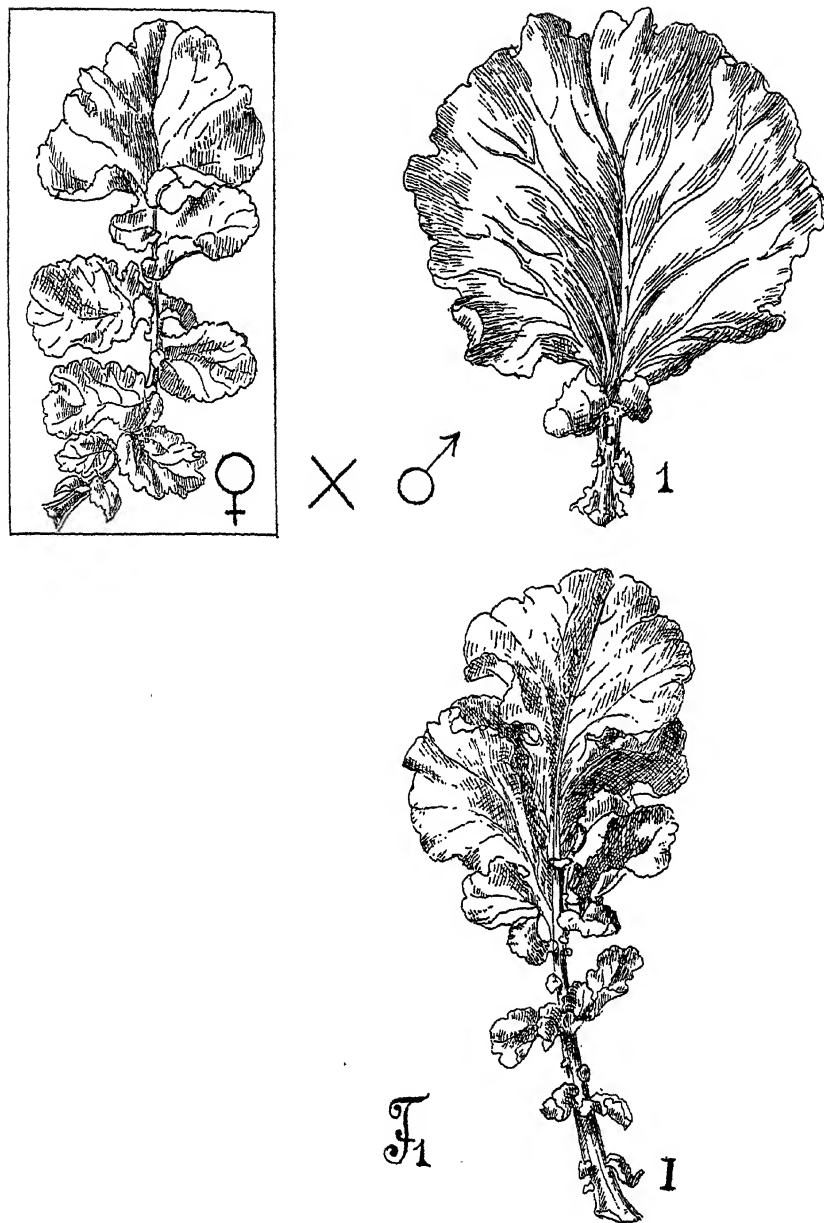
No. of cross-ings	Name of crossed plants ♀ and ♂	Types of $F_1$	No. of hybrids of each type	Height of hybrids in cm.	Size of lower leaves in cm.	No. of hybrids with leaves of an intermediate character				No. of hybrids with leaves like cabbages				No. of hybrids flowering during the 1st year	No. of hybrids with growth on roots
						with a waxy effl.		without a waxy effl.		with a waxy effl.		without a waxy effl.			
						Pubescent	Glabrous	Pubescent	Glabrous	Pubescent	Glabrous	Pubescent	Glabrous		
17 <sup>ae</sup>	<i>R. sativus</i> L. <i>prol. niger</i> Pers. × <i>B. oleracea</i> L. var. <i>capitata</i> L. Radish × hearting cabbage	I II III	9 6	35-90 220-253	8/20-10/28 15/40-18/52	• • •	5 • •	4 • •	• • •	• • •	• • •	• • •	• • •	7 • •	6 • •
23 <sup>ae</sup>	<i>R. sativus</i> L. <i>prol. niger</i> Pers. × <i>B. oleracea</i> L. var. <i>gongylodes</i> L. Radish × Kohlrabi	I II III	3 3 4	5-23 *9-88 230-260	8/26-9/29 17/47-20/68 9/22-13/38	• • •	3 • •	• • •	• • •	• • •	• • •	• • •	• • •	1 • •	• • •
4 <sup>ae</sup>	<i>R. sativus</i> L. <i>prol. niger</i> Pers. × <i>B. oleracea</i> L. var. <i>gongylodes</i> L. Radish × Kohlrabi	I II III	6 2 6	12-38 *10 107-250	10/24-12/33 23/70 7/18-11/39	• • •	• • •	4 • •	1 • •	• • •	• • •	• • •	• • •	2 • •	• • •
16 <sup>ae</sup>	<i>R. sativus</i> L. <i>prol. niger</i> Pers. × <i>B. oleracea</i> L. var. <i>gemmifera</i> D.C. Radish × Brussels sprouts	I II III	5 2 •	8-54 25-90 •	6/16-11/32 18/56 •	• • •	• • •	5 • •	• • •	• • •	• • •	• • •	• • •	1 • •	1 • •

\* Rosettes of leaves, no sprouts.

TABLE III.

Types of $F_1$	Medium height in cm.	Medium size of leaves in cm. br./leng.	No. of hybrids with leaves of the radish type				No. of hybrids with leaves of an intermediate type				No. of hybrids with leaves of a cabbage type				No. of hybrids flowering during their growths 1st year on roots
			with a waxy efflorescence		without a waxy efflorescence		with a waxy efflorescence		without a waxy efflorescence		with a waxy efflorescence		without a waxy efflorescence		
			Pubescent	Glabrous	Pubescent	Glabrous	Pubescent	Glabrous	Pubescent	Glabrous	Pubescent	Glabrous	Pubescent	Glabrous	
I	28.7	9.9/28.2	40	1	•	3	13	•	•	•	•	•	•	•	22
II	48.7	16.0/57.1	52	3	•	18	14	•	•	•	•	•	•	•	36
III	180.4	14.0/38.6	31	•	1	8	6	•	•	•	•	•	•	•	18
Total			123	4	3	29	20	6	7	3	7	•	•	•	76





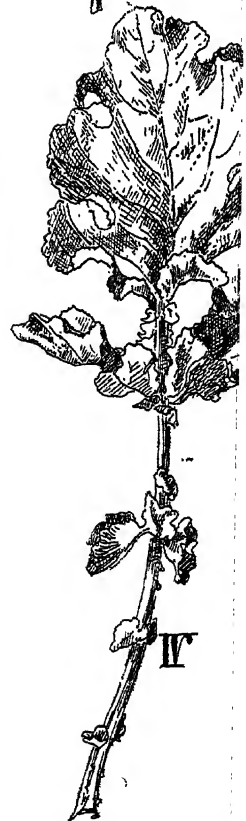
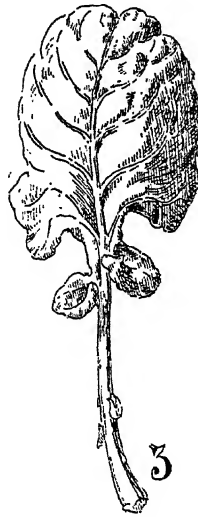
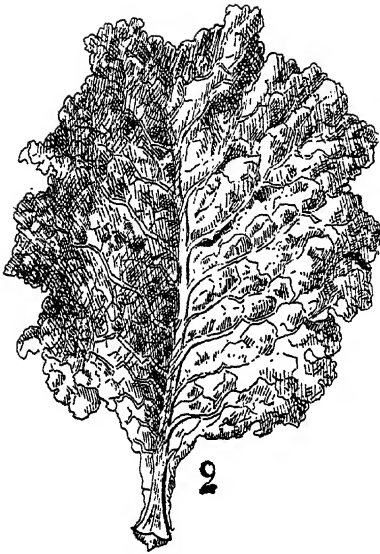
Text-fig. 1.

LOWER LEAVES:

♀ *Raphanus sativus* L. *prol. niger* Persival.

- ♂ {  
 1 *Brassica oleracea* L. v. *capitata* L.  
 2 *Brassica oleracea* L. v. *sabardida* L.  
 3 *Brassica oleracea* L. v. *gemmaifera* D.C.

- F1 {  
 I ♀ *Raph. sat. niger* × ♂ *Brass. ol. capitata* L.  
 II ♀ *Raph. sat. niger* × ♂ *Brass. ol. sabardida* L.  
 III ♀ *Raph. sat. niger* × ♂ *Brass. ol. gemmaifera* D.C.



account of an insufficient development of the conducting system that hollows existed in bulbs of the hybrids of radish × kohlrabi, with numerous additional roots. Other peculiarities in the development of stems of cabbages do not appear in hybrids of radish × cabbages. No hearts were formed, and instead of the small hearts in Brussels sprouts, their hybrids developed only many side branches—dormant buds thus changed into developing ones<sup>1</sup> (see Pl. XXI, fig. 2).

*Roots.* The radish has a thick central root and a cabbage has thin and numerous roots. The hybrids had one central more or less enlarged root, but also many side roots which were freely distributed in the soil, or, perhaps because of a rather prolonged cultivation in garden pots, were tightly twisted around the central root (Text-fig. 2 D). In taste all roots of the hybrids were like radish but less pungent. It is interesting that most hybrids (see Tables I, II and III) had peculiar outgrowths of different shapes and size on their roots. These outgrowths covered sometimes all side roots and gave leaf-bearing shoots, which sometimes appeared on the surface of the soil (see Pl. XXI, fig. 3). Neither radish nor cabbage ever develop such shoots. These outgrowths are of an exogenous origin and consist of a completely solid parenchyma, rich in starch grains and traversed by vascular fibre bunches; the leaflets acquire sometimes, even underground, a differentiation of tissues. These outgrowths were observed on flowering and on non-flowering plants alike, on vigorous and stunted hybrids, and are met in all crosses (see Table II). An analogous development of outgrowths and leaf shoots has been observed before on hybrids of *Brassica Napus* L. × *Brassica Rapa* L. (Lund and Kjaerskou, Wilson, Kajanus). This parallelism, if there is no infection by bacteria<sup>2</sup>, is very interesting in connection with similar hereditary variability in genetically related species (homologous series of variations of Vavilov and Baur).

*Leaves.* The difference between leaves of radish and cabbage is very great. Radish leaves are coarse and hairy, dissected with long unevenly dentated sections. Cabbage leaves are glabrous with a waxy bluish efflorescence; they are more fleshy, dissected in a lyre-form so that their terminal section is much greater than the lateral sections. Hybrids resembled radish or cabbage (seldom) or were of an intermediate type. Intermediate types are shown along with parental forms in Text-fig. 1.

Pubescence or a waxy efflorescence was observed separately and

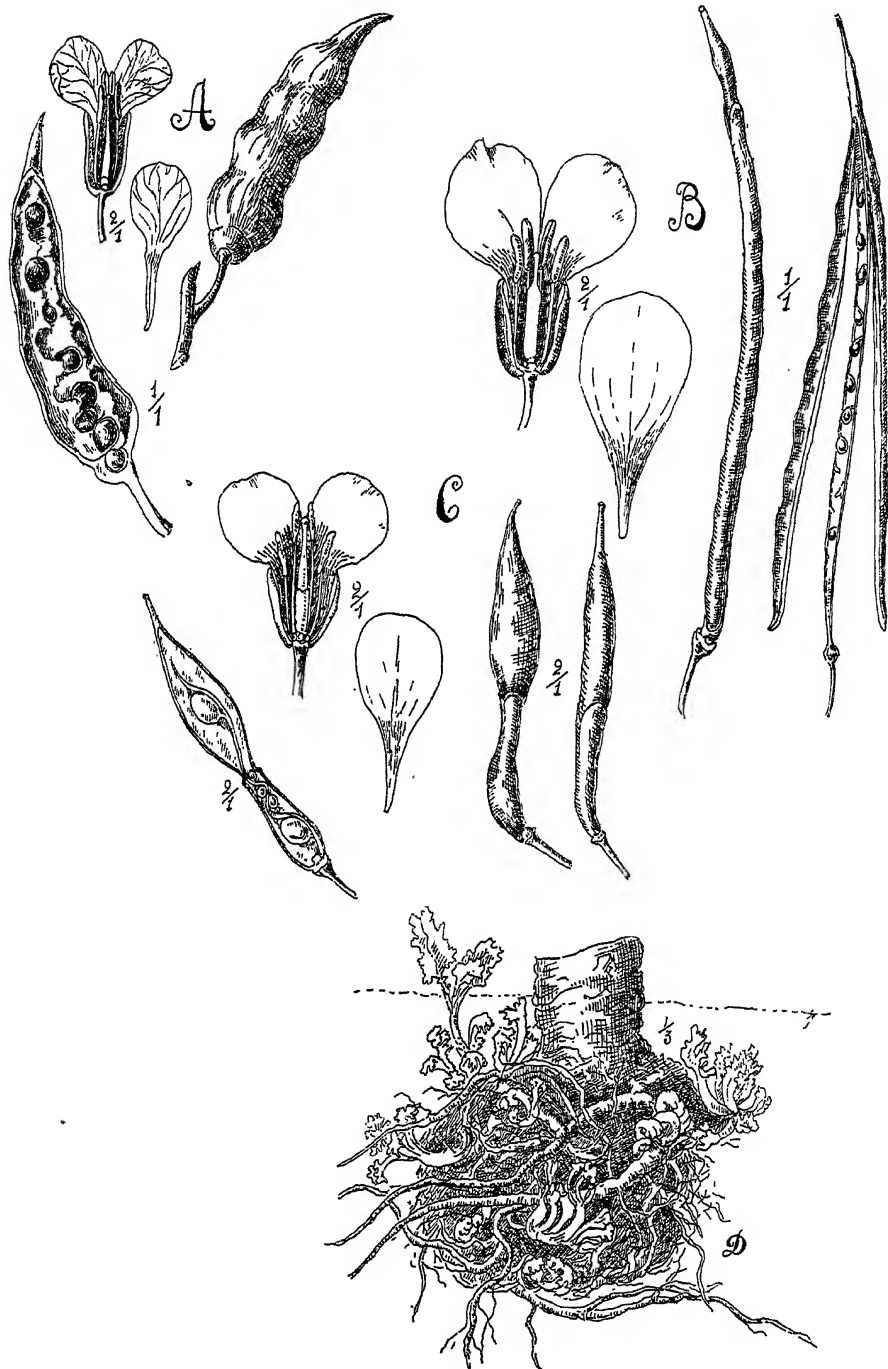
<sup>1</sup> In interracial crossings of cabbages dormant buds and heads are dominant.

<sup>2</sup> Dr Peklo (Prag) found in outgrowths of *Br. Napus* × *Br. Rapa* bacteria which were determined by Kajanus as *Bact. tumefaciens* (1917).

sometimes together, or not at all. Pubescence was seldom observed on leaves of a cabbage type, or a waxy efflorescence on leaves of a radish type. Table II shows that hybrids of a single cross present all possible combinations of parental characters. There is no indication of the degree or strength of the characters in the table: therefore we point out that on cabbage leaves the pubescence was slighter than on radish leaves just as a waxy efflorescence was in them stronger than on radish leaves. The table shows that small plants had almost always leaves of a radish type, hybrids of the 2nd type had intermediate leaves and hybrids of the 3rd type had often real cabbage leaves. This is obvious in Table III where the 123 hybrids are ranged according to their types and leaves. The stunted type is thus linked up with maternal radish leaves, and a vigorous type with cabbage leaves of the parental type. As in interracial crosses, the reddish colouring in crosses of radish  $\times$  red head cabbage dominated, and 3 out of 4 hybrids of radish  $\times$  Savoy cabbage had also crinkled leaves. Hybrids of the 1st type were of a light green colour, and hybrids of the 2nd and 3rd type dark green, but as in general the colour of leaves changes easily its exact definition is difficult.

*Flowers.* The flower-clusters of radish and cabbage have on the average about 20–40 flowers. Clusters of hybrids of type I had 15–35 flowers and hybrids of type III developed in separate clusters as many as 170–190 flowers and had still other flower-buds. But simultaneously no more than 7–10 flowers were in bloom, the rest fell and they could be counted only by their stems. Probably this abundant flowering was due to the fact that only a few pods were set. The calyx in radish is pubescent, of the cabbage smooth like those of the hybrids. Petals of radish are about 1.5–1.8 cm. long generally, and white with dark nerves or purple. Petals of cabbages are 2–2.3 cm. long, yellow. Their flowers are shown on Text-fig. 2 *A* and *B*. Petals of hybrids were of 1–2 cm. in length of an intermediate shape (Text-fig. 2 *C*) and of various colours: white, purple or of a pale-cream colour (one plant)<sup>1</sup>. Hybrids and parents were analysed by N. E. Prokopenko in order to determine the presence of flavone and anthocyanin. The presence of either pigment was determined according to a change in colour of petals in vapours of ammonia: anthocyanin changes into blue, flavone into yellow, a mixture of both changes into green. Petals of radish appear to possess both flavone and anthocyanin and the last one is present in nervures espe-

<sup>1</sup> In crosses of *Raphanus sativus* L.  $\times$  *Raphanus raphanistrum* L. white colour dominates or prevails (Focke).



cially; the cabbage possess only flavone in petals and nervures alike. Hybrids are different and sometimes peculiar, e.g. one hybrid of radish  $\times$  hearting cabbage had in all flowers flavone only in nervures and at the base of the claw of petals and anthocyanin only in a little spot at the outer end of petals. Parents of this hybrid did not show any similar distribution. Gravatt, as we said, found eight stamens in some flowers of his hybrid. Such changes did not occur in our cases<sup>1</sup>: like all Cruciferae they had six stamens, their pollen was not powdery but stuck together, and it was difficult to use it when crossing.

The ovaries of radish consist of two segments: a lower small one containing a single ovule, and a long upper one gradually passing into the pistil—with 8–12 ovules. Cabbages have one single ovary with 30–35 ovules, and pass directly into the pistil. The hybrids have 2-celled ovaries but, in contrast to the radish, both segments were equally developed and had 3–6 ovules: the upper segment gradually narrowing into the pistil (see Text-fig. 2 C). As in the parental forms there is a longitudinal partition; the kind of ovaries determines the shape of the pods.

*Pods.* Radish has a spindle-like, inflated pod which does not split and consists of two parts, of a lower, smaller one, often sterile or containing one seed only and an upper one enlarged by a spongy mesocarp contracting towards the end, more or less narrow between the seeds (see Text-fig. 2 A). Cabbages have a linear pod splitting into two valves and elongated into a sterile or one-seeded apex (see Text-fig. 2 B). Our hybrids had no ripe seeds. Most of the ovaries fell off soon after pollination and only some, especially in hybrids of radish  $\times$  hearting cabbage “kubyshka,” developed for some time. Text-fig. 2 C shows young hybrid pods magnified twice: they are very peculiar as their lower part is distinctly 2-celled like a cabbage pod, but the upper part is more like a radish pod. An anatomical study of these hybrid pods shows that the longitudinal partition is pushed aside towards one or other wall of the pod, and goes over into the upper part; the sponge-like mesocarp develops in the upper part of the pod, but occurs also in the lower part. The resemblance to radish or cabbage pods depends upon the development of seeds in the lower or upper part of the pod; but the structure of pods is alike in all hybrids, and they would be considered by a systematist as a definite independent form. The difference of the pods in Sageret’s case was probably due to their outward appearance only.

<sup>1</sup> Only one flower in a hybrid was abnormal, having six petals in calyx, six petals, seven stamens and a deformed pistil.

## 386 *Hybrids of* ♀ *Raphanus sat. L.* × ♂ *Brassica ol. L.*

The morphology of the pods of the hybrids seems clear: botanists like Pomel, Hayek, Schulz and others who have studied Cruciferae believe that the small segment of the pod in *Raphanus* is due to a reduction of a two-valved pod (*Valvarghied*) and the pod of radish is in reality only a developed manyseeded apex (*Stylarghied*). In *Brassica oleracea*, conversely, the two-valved part is developed and the apex remains small. Hybrids naturally receive both characters and we have really a pod where both segments are equally developed. Thus hybridisation confirms the conceptions of morphologists—a method rarely used till nowadays.

### *Conclusion of the morphological description of hybrids.*

Thus a description of hybrids of *Raphanus sativus* L. × *Brassica oleracea* L. shows that  $F_1$  is quite uniform as to the form of pods and flowers but differs in form, pubescence, waxy efflorescence of leaves and in colour of flowers, while in habit of growth and vigour it is divided into two or three types. *Raphanus sativus* L. and *Brassica oleracea* L. are cross-fertilizers and therefore heterozygous;  $F_1$  in their interracial crossings is always polymorphic, thus cabbages which were crossed with radish in 1922 were also intercrossed with each other and their  $F_1$  was different in shape and leaves and length of stem. But in separate crosses the difference among racial hybrids of cabbages or radishes is insignificant and quite unlike that found in the generic crosses of *Raphanus* × *Brassica*. Their striking divergence in some characters and the habit of growth is probably due not only to their parents having been heterozygous but also to the peculiar nature of hybrids. We have here in one organism two quite different haploid collections of chromosomes, whose gens are mostly different and do not pair up with each other; they give no allelomorphs and act independently and separately. Thus no dominance or recessiveness can be observed and the heterozygosis of the parental forms is obvious, but at the same time the normal development of characters and of the whole organism is injured. The morphology of hybrids is determined in this case not only by gens but also by their more or less harmonious action. A further study of the hybrids is required for a detailed determination of their morphogenesis. The question about the divergence in  $F_1$  is very complicated and requires great caution in its solution<sup>1</sup>.

<sup>1</sup> For various theories see Atkinson, "Sorting and blending of unit characters," *Zeit. f. ind. A.* 1916. Bd. xvi.

CYTOLOGY OF HYBRIDS<sup>1</sup>.*The nuclear division in somatic cells of hybrids.*

Most of the hybrids of the stunted type, if they did not flower, differed from radish only by their weaker development, by their more branched roots and by outgrowths on them. It was natural to inquire: are not the cabbage chromosomes eliminated in them under an influence of an unfamiliar protoplasm? A similar phenomena has been observed by Baltzer in fertilization of eggs of a sea-urchin of the genus *Strongylocentrotus* by sperm of the genus *Sphaerechinus*; chromosomes of the foreign spermatozoid were thrown out of the spindle and did not get into the daughter cells. Nor did they participate in the further development of the egg, which reached the Pluteus state, and had a purely maternal type of skeleton.

I have studied the nuclear division in cells of the root-tip and of stem-shoots of two radish × cabbage hybrids of the stunted type, most alike to radish, and of one hybrid of the vigorous type, intermediate in its morphological characters. It appeared that the division was normal in all three hybrids. According to my investigations in 1922, *Raphanus sativus* L. and varieties of *Brassica oleracea* L. have a diploid number of chromosomes equal to 18 and their haploid number is thus 9. The hybrids in somatic cells have 18 chromosomes, the sum of the haploid numbers of their parents. Thus there is no diminution in numbers of chromosomes or abnormalities in divisions of nuclei in somatic cells of the hybrids.

*The reduction of the chromosomes in maternal cells of pollen.*

The reduction of the chromosomes in anthers of *Raphanus sativus* L., *Brassica oleracea* L. and their hybrids can be observed in small buds about 2 mm. in length fixed when the weather is warm and favourable for growth. The time is of no importance as I found heterotypic division in materials fixed alike in the morning, during the day, and in the evening. In preparations of such buds one sees ordinarily synapsis and tetrads of pollen, but sometimes also other stages of heterotypic mitoses are seen which are evidently proceeding with greater rapidity. In the

<sup>1</sup> The material for cytological investigations was fixed by a mixture according to S. G. Navaschin: 15 parts of 1 per cent. of chromic acid + 1 part of glacial acetic acid + 3 parts of 16 per cent. of formalin + 17 parts of dist. water. Microtome sections were 5-10 $\mu$  thick; for staining iron haematoxylin was used.



two outer, shorter anthers the division begins later than in the four inner, longer anthers (e.g. when in the shorter anthers the first division takes place, in the longer one the pollen tetrads are already formed). Separate nests of anthers have also sometimes different stages of division, although in closer limits; they are later in upper parts of the nest. The reduction of the chromosomes proceeds alike in *Raphanus sativus* L. and *Brassica oleracea* L.<sup>1</sup> This division is shown in Figs. 1–12 of Pl. XXII, made from preparations with Abbé's drawing apparatus. It is difficult to say anything definite about the behaviour of chromatin threads in the synapsis (Fig. 1) because of the minuteness of the nuclei in *Raphanus* and *Brassica*. At first thin and long, these threads get contracted, thicker, and at last fall into separate segments or chromosomes, which lie in pairs (Fig. 2). There appears in the synapsis besides the first one, also another nucleolus, which is less intensively coloured by haematoxylin and is almost always near the first one as if "coming out" of it.

The next stage after synapsis is diakinesis when in *Raphanus sativus* L. (Fig. 4) and in *Brassica oleracea* (Fig. 3) alike a full conjugation of the pairs of chromosomes takes place, resulting in a formation of nine "bivalent chromosomes." The nucleoli sometimes are still there, or disappear. The nuclear membrane gets dissolved and the chromosomes are distributed in one plane. The metaphase of the first division then begins. If the spindle in this stage is seen from above (from the pole) the chromosomes are quite round and can be easily counted (Figs. 5 and 6), but if seen sidewise one can observe only the equatorial plane of the chromosomes (Fig. 7). Here a half of the bivalent chromosomes divides from each other, and later in anaphases of the first division (Fig. 8) a quite regular parting of them takes place towards the poles, nine to each pole. The parted chromosomes build daughter nuclei in the stage of the telophase (Fig. 9): a nucleus cover appears again and nucleoli are formed. But there is no resting stage, as the telophases of the first division pass into prophases of the second division: the nucleoli disappear and the chromosomes become twisted like 8 (Fig. 10). The nuclear membranes are again dissolved and in the metaphase of the second division two spindles are built in different planes (Fig. 11); the chromosomes split, and their halves proceed towards the poles, building four nuclei placed in a tetrahedron. Again cell membranes testiales appear and four separate cells emerge (Fig. 12)—the tetrads of pollen developing later on into pollen grains. Thus the heterotypic mitoses in *Raphanus*

<sup>1</sup> I studied *Brassica oleracea* L. var. *sabauda* L.

*sativus* L. and *Brassica oleracea* L. proceed quite normally according to the scheme usual for *Dicotyledonae*<sup>1</sup>.

The reduction of the chromosomes has been observed by me in anthers of six hybrids: in four hybrids of radish  $\times$  kohlrabi and in two of radish  $\times$  hearting cabbage; in all plants the figures of division were alike. In synapsis (Pl. XXII, fig. 13) the hybrids do not differ from the maternal forms; but later on the spireme of chromatin threads divides into chromosomes (Fig. 14) which do not conjugate during diakinesis but remain lying separate and disorderly along the periphery of the nucleus (Figs. 15 and 16). Whereas in the parental forms we have in diakinesis nine bivalent chromosomes, in hybrids because of an absence of conjugation, there are nine radish and nine cabbage univalents or 18 chromosomes (Figs. 3, 4, 15 and 16). Sometimes one observes 17 or 16 chromosomes but there are no typical "gemini," and some chromosomes are simply hidden behind others or are covered by the nucleolus. As the nuclei are very small and the chromosomes are heaped together, this is very probable.

This absence of conjugation of parental chromosomes is also evident in the next stage, the metaphase of the first division. The parent plants have in this stage 9 big bivalent chromosomes but the hybrids have 18 small univalent chromosomes, corresponding in size to chromosomes of the anaphase of the parents (Fig. 17, compare with Figs. 5 and 6 with Fig. 8). The form of chromosomes in the hybrids is less definite than in *Raphanus* and *Brassica*. The chromosomes do not form in metaphases normal nuclear plates, although they are heaped in cells more or less; some of them are sometimes seemingly thrown towards the very borders of the cell (Figs. 17, 18). In the side view of the metaphase the hybrids have no spindles of division, and only sometimes one observes separate fibres (Fig. 19, compare with Fig. 7). There are no typical anaphases—the chromosomes proceed towards the poles irregularly (Fig. 20). The telephases have often in the beginning the form of 8 (Fig. 21) and then the daughter nuclei are formed (Fig. 22). An unequal number of chromosomes is used: from 6 till 12. Separate chromosomes and those which remain behind during the separation to the poles—often do not get into the daughter cells and remain included in the plasma as separate inclusions of chromatin. In some cases chromo-

<sup>1</sup> Rarely one observes in *Brassica* such anomalies as e.g. a separation of a pair of chromosomes in the anaphase from the others which travel to the poles. These separated chromosomes remain in the protoplasm and do not participate in the formation of the nuclei.

somes did not separate at all during the first division and formed one big nucleus (Fig. 23).

Already in the telophases the second division begins—a longitudinal splitting of chromosomes (Fig. 24). In the metaphase of the second division a normal development of spindles is often observed as also regular metaphases with a different number of chromosomes (Fig. 24). In anaphases chromosomes are distributed unevenly, some of them are often thrown out altogether from the sphere of division (Figs. 25, 26), the spindles are long and not always rightly adjusted; sometimes we observed a fusion, as it were, of the two spindles (Fig. 27). Telophases have often a form of a tetrahedron, but with outward inclusions of chromatin (Fig. 28). A precise determination of the number of chromosomes in anaphases and telophases of the 2nd division is impossible because of their accumulation. The protoplasm in pollen mother cells of hybrids is quite normal.

The hybrids have therefore besides normal pollen tetrads (Fig. 29) groups of two, three and even seven cells (Figs. 30, 31). The cells are not alike in form and size, they have nuclei of different dimensions, and some of them contain chromatin only in separate grains.

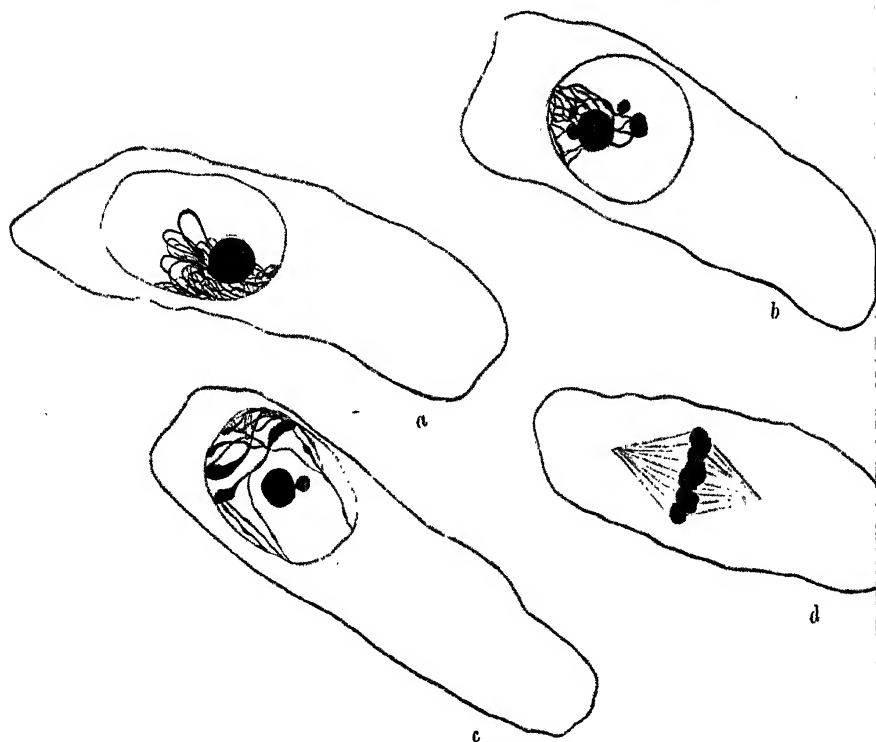
Young pollen developed even out of small cells grows, but later its contents degenerate, the protoplasm and nuclei disappear and only wall membranes remain. But some pollen grains are preserved and they develop. The first division in pollen takes place (Fig. 32), generative cells are separated, and in some cases even a division of the generative nucleus (Fig. 38) has been observed as well as perfectly mature pollen with two male nuclei (Fig. 34). The protoplasm in such pollen is quite normal.

Thus we observe definite anomalies in the process of heterotypic division of pollen-mother-cells—in hybrids of radish × cabbage, proper to all hybrids of distant forms. A complete absence of conjugation of parental chromosomes in diakinesis was first observed by Federley in interspecific crossings of the moth *Pygaera*. The author could not find in them the stage of synapsis, and the univalents behaved differently from those in hybrids of *Raphanus* × *Brassica*: they split in the first and second division and their halves were evenly distributed so that each spermatozoid had a diploid or nearly diploid number of chromosomes<sup>1</sup>. Chromosomes did not conjugate also in the prophase of a

<sup>1</sup> Only separate chromosomes conjugated sometimes in diakinesis. An absence of conjugation observed also Doncaster and Harris in hybrids of butterflies *Bistoninae*. *Journ. of Genetics*, 1914.

heterotypic division according to Poll's studies of hybrids of pea-fowls with guinea-hens.

In plants this phenomena was first observed by Haase-Bessel in interspecific hybrids of *Digitalis purpurea* × *Digitalis lutea*. Hybrids of *Raphanus sativus* L. × *Brassica oleracea* L. are in this respect a second example. Anomalies in the development of both hybrids are almost alike.



Text-fig. 3 (a-d). Reduction division in embryonic mother cell. a. Synapsis in *B. ol.* L. b. Synapsis in hybr. of *R. sat.* L. × *B. ol.* L. var. *capitata* L. c. Deployment of the synapsid knot in hybr. of *R. sat.* L. × *B. ol.* L. var. *capitata* L. d. Metaphase of the first division in hybr. of *R. sat.* L. × *B. ol.* L. var. *gongyloides* L.

*The reduction of the chromosomes in the ovules.*

The reduction in the ovule of hybrids of *Raphanus sativus* × *Brassica oleracea* begins in buds of 3-4 mm. in length like that of the parental forms, i.e. when tetads are developed in anthers and young pollen is built<sup>1</sup>. Synapsis in the ovule of hybrids has been observed by me several times (Text-fig. 3 a and b), but the deployment of the chromatin knot

<sup>1</sup> *Brassica oleracea* f.e. is decidedly protogynous.

proceeds anomalously (Text-fig. 3 c). Out of a great number of ovaries I found only once a very clear preparation with a metaphase of the first division, where it was proceeding quite normally—there was a normal spindle and one could see in the equatorial plate nine bivalent chromosomes (Text-fig. 3 d). In the same bud pollen was quite anomalous. No other stages of heterotypic division could be found, and I never observed the development of an embryo sac. Possibly the development of the megaspore mother cell ceases in hybrids in the stage of the prophase or after the parting of chromosomes—in the anaphase of the first division. A different behaviour of chromosomes in anthers and ovules was pointed out by Rosenberg for *Hieracium Pilosella*, by Täckholm for the *Rosa canina* group, and by Overeem for hybrids of *Oenothera gigas* × *Oenothera semigigas*. In previously mentioned studies Federley and Poll investigated only spermatogenesis, and Hasse-Bessel observed in *Digitalis* during diakinesis an absence of conjugations of chromosomes in ovaries and anthers alike.

Cases of different conjugations of chromosomes in the development of male and female gametes, the influence of external factors (see Tischler), and new data of Federley (1917) showing that racial hybrids of some butterflies do not differ in behaviour of chromosomes from interspecific and generic hybrids, may possibly indicate that the conjugation of chromosomes does not depend on gens but on some other causes.

*Sterility of hybrids and reciprocal crossings of ♀ Brassica oleracea L. × ♂ Raphanus sativus L.*

Sageret's statement that radish × cabbage hybrids set seeds was not confirmed by Gravatt and Baur. My numerous intercrossings of hybrids among themselves and with the parental forms gave no results. The ovaries sometimes developed did not contain any embryos. Experiments with germination of pollen in various solutions all failed (Gravatt).

Sterility of gametes in hybrids is probably due to an occasional distribution of parental chromosomes during the reduction of the chromosomes, why most gametes never get a haploid collection of chromosomes, necessary for their development.

Cases when active parental gametes may be formed may be too rare to be observed in experiments. Possibly during a prolonged vegetative propagation of hybrids they may acquire some fertility as observed by Wettstein in *Sempervivum* hybrids. No reciprocal crossings of *Brassica oleracea* L. × *Raphanus sativus* L. did succeed, as shown by attempts of Sageret, Herbert, Gravatt, Baur and my attempts of 1923. I crossed

also *Brassica oleracea* L. with *Raphanus raphanistrum* L. and *Raphanus odessoinus* Spreng. but without results.

Anatomical investigations of ovaries of cabbages pollinated by radish pollen and fixed on the 3rd or 5th day after do not disclose any development of embryos in ovules; only destroyed embryo-sacs are seen in preparations. Pollen on stigmas and in pistils gives only short tubes, or does not germinate at all. Evidently this failure is due to physiological peculiarities of the stigmas of cabbages, hindering a normal germination of radish pollen.

#### SUMMARY.

In summarising the above data one has to state that a detailed study of generic hybrids of ♀ *Raphanus sativus* L. × ♂ *Brassica oleracea* L. establishes some new facts of a general genetical interest. These facts can be enumerated as follows:

1. Hybrids of  $F_1$  of *Raphanus sativus* × *Brassica oleracea* differ much among themselves in vigour, habit of growth, in leaves and in colour of flowers. In separate crossings occur exceptionally vigorous and also stunted plants, the latter being mostly of a maternal character.

2. This polymorphism of hybrids does not affect the structure of flowers and fruits. Those characters are intermediate for all hybrids.

3. The roots of hybrids have outgrowths and leaf shoots just as hybrids of *Brassica Napus* L. × *Brassica Rapa* L.

4. The nuclear division in somatic cells (somatic mitoses) proceeds quite normally.

5. The heterotypic mitosis in pollen-mother-cells is quite anomalous: the parental chromosomes do not conjugate in diakinesis, and their distribution during the first and second division is anomalous; besides the pollen-tetrads, groups consisting of two, three and even seven cells are formed.

6. Of the grains of pollen only a few are developed, and pollen mostly degenerates.

7. In cells of archesporium the early stages of a meiosis were observed: synapsis and the dissolution of the knot of chromatin threads; once a normal metaphase of the first division was observed with a normal spindle and bivalent chromosomes. Other stages of division were not observed. No formation of embryo-sacs in ovules of hybrids could be detected.

## 394 *Hybrids of* ♀ *Raphanus sat. L.* × ♂ *Brassica ol. L.*

8. Hybrids of *Raphanus sativus* L. × *Brassica oleracea* L. remain sterile when intercrossed or crossed with parental forms.

9. A reciprocal crossing between ♀ *Brassica oleracea* L. × *Raphanus sativus* L. ♂, does not succeed.

I have to acknowledge and to express my gratitude for kind help and interest in my work to Prof. S. J. Jegalov, to A. G. Nikolaeva and U. N. Sveshnikova. I am also much indebted to Prof. S. G. Navaschin for his valuable suggestions and to Prof. N. J. Vavilov for his help in publishing this article.

### EXPLANATION OF PLATES.

PLATE XXI. Fig. 1. General view of the whole collection of hybrids: *Raphanus sativus* L. × *Brassica oleracea* L. In the foreground is a hybrid of the 1st type; then is a series of hybrids of the 2nd type; the left row and one plant at right are hybrids of the 3rd type (tallest). Photographed in October, 1923.

Fig. 2. Hybrids: *Raphanus sativus* L. × *Brassica oleracea* L. var. *sabauda* L. (at right), *Raphanus sativus* L. × *Brassica oleracea* L. var. *gemmifera* D.C. (in the centre), and *Raphanus sativus* L. × *Brassica oleracea* L. var. *capitata* L. (at left). Photographed in October, 1923.

Fig. 3. Outgrowths and leaf shoots on roots of hybrids *Raphanus sativus* L. × *Brassica oleracea* L.

PLATE XXII. The drawings have been made with Abbé's drawing apparatus.

Figs. 1-31 on Pl. XXII and Text-fig. 3 were done with Zeiss, 1/12 homog. immer. + compens. ocular 12.

Figs. 32-34, Pl. XXII at 1/12 homog. immer. + compens. ocular 8.

On reproduction the figures were ×  $\frac{3}{2}$ .

Figs. 1-12. Reduction division in pollen-mother-cells in *Brassica oleracea* L. and in *Raphanus sativus* L.

Fig. 1. Synapsis in *B. ol.* L.

Fig. 2. The first stages of diakinesis in *B. ol.* L.

Fig. 3. Diakinesis in *B. ol.* L.

Fig. 4. Diakinesis in *R. sat.* L.

Fig. 5. Metaphase of the first division in *B. ol.* L.

Fig. 6. Metaphase of the first division in *R. sat.* L.

Fig. 7. Metaphase of the first division in *B. ol.* L. (viewed from the side)

Fig. 8. Anaphase of the first division in *B. ol.* L.

Fig. 9. Telophase of the first division in *B. ol.* L.

Fig. 10. Prophase of the first division in *B. ol.* L.

Fig. 11. Metaphase of the second division in *R. sat.* L.

Fig. 12. Pollen-tetrads in *B. ol.* L.

Figs. 13-31. Reduction division in pollen-mother-cells of hybrids of *R. sat.* L. × *B. ol.* L.

Fig. 13. Synapsis in hybrid of *R. sat.* L. × *B. ol.* L. var. *capitata* L.

Fig. 14. The beginning of diakinesis in hybrid of *R. sat.* L. × *B. ol.* L. var. *capitata* L.

Figs. 15, 16. Diakinesis in hybr. of *R. sat.* L. × *B. ol.* L. var. *capitata* L.

Figs. 17, 18. Metaphases of the first division in hybr. of *R. sat.* L. × *B. ol.* L. var. *gongyloides* L.

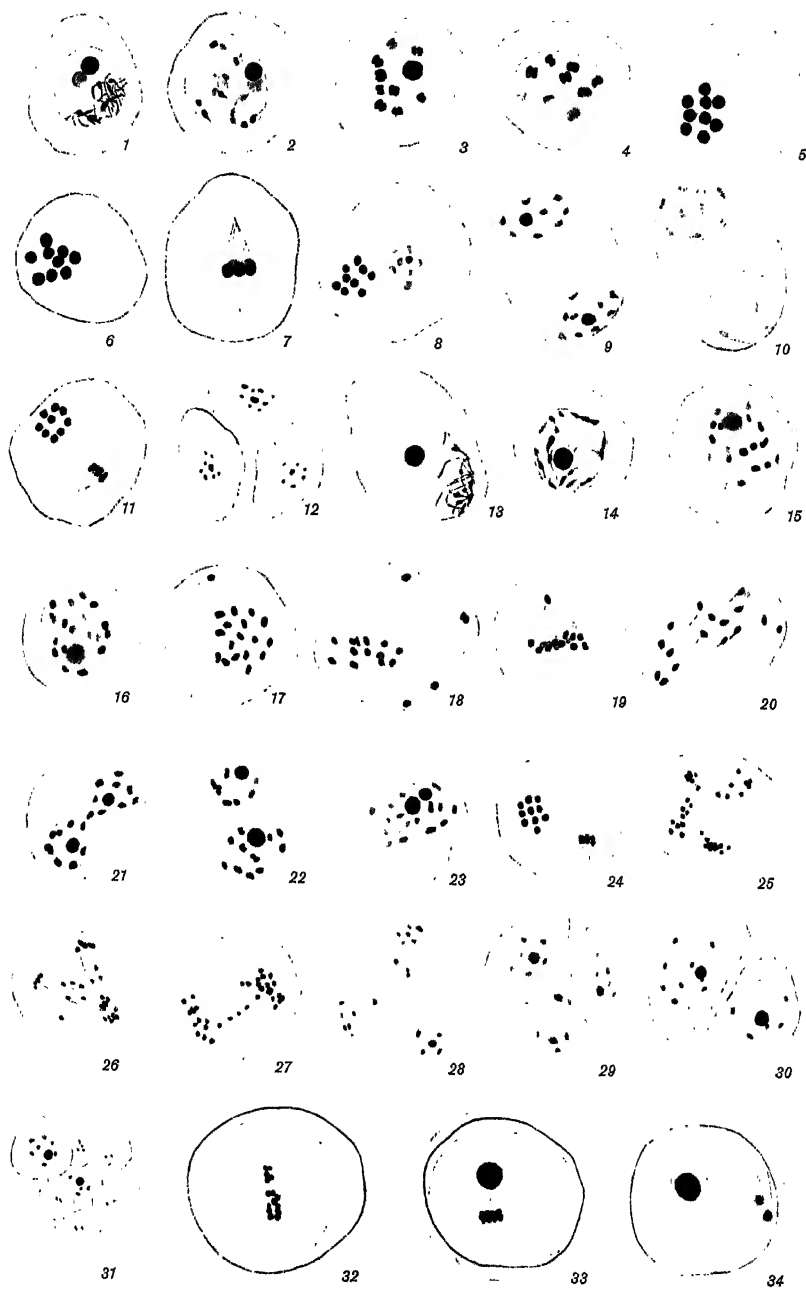


Fig. 1.











- Fig. 19. Metaphase of the first division in hybr. of *R. sat.* L.  $\times$  *B. ol.* L. var. *capitata* L.  
 Fig. 20. Anaphase of the first division in hybr. of *R. sat.* L.  $\times$  *B. ol.* L. var. *gongyloides* L.  
 Fig. 21. The beginning of telophase of the first division in hybr. of *R. sat.* L.  $\times$  *B. ol.* L. var. *capitata* L.  
 Figs. 22, 23. Telophase of the first division in hybr. of *R. sat.* L.  $\times$  *B. ol.* L. var. *capitata* L.  
 Fig. 24. Metaphase of the homoeotypical division in hybr. of *R. sat.* L.  $\times$  *B. ol.* L. var. *capitata* L.  
 Figs. 25, 26, 27. Anaphases of the homoeotypical division in hybr. of *R. sat.* L.  $\times$  *B. ol.* L. var. *capitata* L.  
 Fig. 28. Telophase of the homoeotypical division in hybr. of *R. sat.* L.  $\times$  *B. ol.* L. var. *capitata* L.  
 Figs. 29, 30, 31. Stage of the "pollen-tetrads" in hybr. of *R. sat.* L.  $\times$  *B. ol.* L. var. *gongyloides* L.  
 Figs. 32-34. Pollen grains in hybr. of *R. sat.* L.  $\times$  *B. ol.* L.  
 Fig. 32. The first division in pollen grain in hybr. of *R. sat.* L.  $\times$  *B. ol.* L. var. *gongyloides* L.  
 Fig. 33. The second division in the pollen grain in hybr. of *R. sat.* L.  $\times$  *B. ol.* L. var. *gongyloides* L.  
 Fig. 34. Normal pollen grain with two male nuclei in hybr. of *R. sat.* L.  $\times$  *B. ol.* L. var. *gongyloides* L.

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# VARIATION AND SPECIES IN *CYANOPHYCEAE*<sup>1</sup>.

By W. B. CROW, M.Sc., Ph.D. (LOND.).

*Lecturer in Botany, University College of South Wales  
and Monmouthshire.*

(With Eight Text-figures.)

## INTRODUCTION.

THAT the various characters of an organism are not all of the same value in classification is one of the best known principles of systematic biology. If species are genetically related the possibility exists that they may be grouped according to their relationships rather than their superficial resemblances. The idea of the so-called natural or phylogenetic system transferred the attention of systematists from the more obvious to the less obvious characters, the latter being taken as more indicative of relationship than the former, inasmuch as they were less subject to variation during the course of descent. Thus, although it is uncertain how far it will ever be possible to establish a satisfactory classification on phylogenetic relationship alone, the principle stated above is very generally applied in taxonomy. It has been recognised that some characters are more or less constant throughout large groups of animals or plants, whilst others are confined to a more restricted series of species or even individuals.

In a former publication<sup>(13)</sup> the present writer attempted an analysis of the characters of some of the *Chroococcaceae*, a family of Protista remarkable for their extreme morphological simplicity, with a view to determining the relative importance of the characters in classification. The main object of the present communication is to set forth some results bearing on the relation of the systematic differences to variation in general which a continuation of the above investigation has led to.

Among the higher organisms the phenomena of Mendelism show that sometimes characters may be regarded as units since they may be inherited in sexual reproduction independently of other characters.

<sup>1</sup> This name (Sachs, 1874) is the least confusing of the many that have been applied to the group. The term *Myxophyceae* was used in 1860 by Sittzenberger in the same sense and thus has priority. This latter group name, however, had been applied by Wallroth in 1833 to what now appears as a heterogeneous assemblage of organisms.

Mendelian segregation makes it convenient to deal with the characters of an organism as if they were definite units or, in the complicated and more usual cases, as if they were due to definite unit factors. In the following pages it will be necessary to use the term character somewhat frequently. The term is, however, not used in the restricted Mendelian sense. The present work is a study of specific rather than individual variation. As in the investigation of the differences between individuals, it will be convenient to deal independently with the characters by which one species differs from another or to reduce them to factors which may be treated independently. But we shall find that the specific distinctions differ in important respects from Mendelian variations.

The systematic characters of the *Cyanophyceae* at present recognised are comparatively few and simple. A description and classification of these characters will first be attempted.

#### INTERNAL STRUCTURE OF ARCHIPLAST.

The cytological organisation of the *Cyanophyceae* is now generally recognised to differ from that of other groups. The term archiplast is

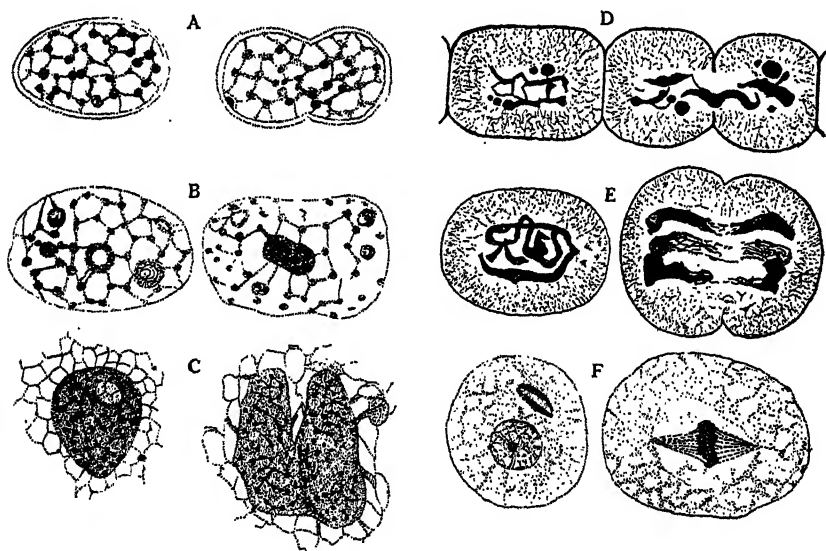


Fig. 1. Types of cytological structure in five members of the *Cyanophyceae* and in one member of the *Chlorophyceae* (*Tetraspora*). In each the undivided and the dividing archiplast or cell is shown. A. *Aphanothece prasina* after Acton. B. *Merismopedia elegans* after Acton. C. *Chroococcus macrococcus*, central portion of archiplast only, after Acton. D. *Cylindrospermum licheniforme* after Gardner; figure modified by omission of an archiplast. E. *Synechocystis aquatilis* after Gardner. F. *Tetraspora lubrica* after McAllister.

due to Nadson<sup>(29)</sup> and has also been used by G. S. West<sup>(38)</sup>. It indicates the protoplasmic unit of these forms as a lower and probably more primitive type than the ordinary algal cell. But apart from this general difference there is, as in many classes of Protista, a considerable variation amongst the different species (Fig. 1). This was demonstrated by the work of Acton<sup>(1)</sup> and of Gardner<sup>(21)</sup>. The occurrence of a characteristic central body may be constant for a given species<sup>(13)</sup>. The presence of different types of granules is not generally sufficiently constant to be of significance, but the granulation of the transverse walls in the filaments of *Oscillatoriaceae* may be cited as being regarded by some authorities as of systematic value. Gomont<sup>(23)</sup> describes the genus *Spirulina* as constantly showing homogeneous contents. The presence of gas vacuoles may also indicate systematic position in some forms<sup>(13)</sup>.

#### MODE OF DIVISION.

Multiplication by fission takes place by definite planes of division which generally remain constant in each species. In the unicellular forms (*Chroococcaceae*) this may be in one plane only, when the archiplast is generally elongated, and it may be perpendicular to the major axis (*Gloethece*) or oblique to it (*Dactylococcopsis*) or in two or three planes at right angles; in filamentous forms one plane of division only is the predominant condition. In most cases this is transverse, but in the *Oscillatoriaceae* and *Scytonemaceae* oblique divisions are also observed<sup>(8)</sup>.

Branching, which might equally well be regarded as incomplete division, is dealt with below, as a modification of the form of the trichome or the colony of trichomes.

The division of the filaments of the *Oscillatoriales* by ingrowing septa into segments must be distinguished from actual division of the filament into independent offspring filaments. The former process does not seem to vary amongst the species.

#### PIGMENTATION OF ARCHIPLAST.

The pigmentation of the archiplast varies independently of its internal structure. Whilst more subject to variation with external conditions it is, however, sometimes the only difference between forms otherwise similar and remains constant in these, even when growing in identical habitats. The colour differentiation of the different species belonging to the genus *Oscillatoria* may be cited as an example. Among unicellular forms the species of *Gloeocapsa* exhibit a wide range of colour variation accompanied by a very uniform morphology. The occurrence of forms apparently



identical with species of various genera of *Cyanophyceae*, except in the violet colouration of the cells, is an example of the isolated occurrence of a similar character at various points in the system.

#### FORM AND DIMENSIONS OF ARCHIPLAST OR TRICHOME.

##### TRUE BRANCHING.

In the unicellular *Cyanophyceae* the protoplasmic unit can be considered apart from the surrounding envelope. It has long been customary to describe the series of protoplasmic segments of a filamentous species independently of the surrounding sheath. The term trichome is restricted to the former, the term filament including both trichome and sheath.

The proportion of plasma to envelope varies. In both unicellular and

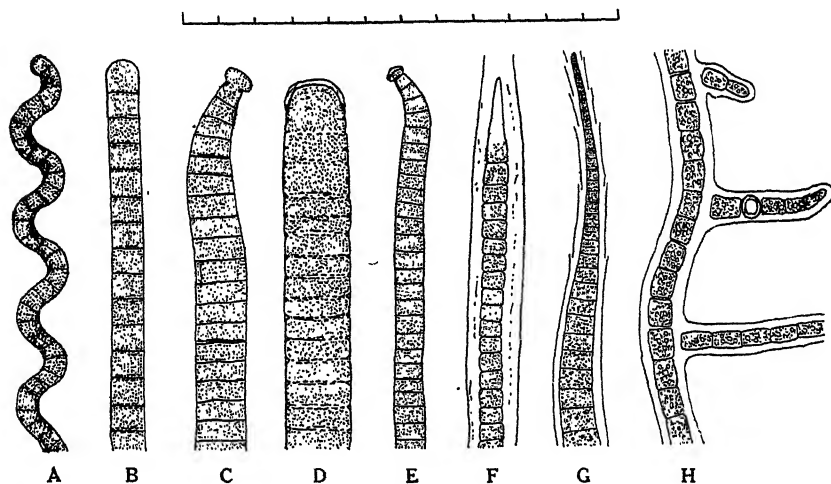


Fig. 2. Types of trichome in *Cyanophyceae*. A. *Arthrospira Jenneri*. B. *Oscillatoria irrigua*. C. *Oscillatoria proboscidea*. D. *Oscillatoria margaritifera*. E. *Phormidium uncinatum*. F. *Schizothrix purpurascens*. G. *Calothrix parietina*. H. *Hapalosiphon arboreus*. Each scale division measures 10 microns.

filamentous forms the latter may be practically absent (*Synechocystis*, *Oscillatoria*) whilst in species having archiplasts or trichomes which are no larger than in these forms, the envelope may be well developed, attaining a thickness several times greater than that of the enclosed archiplast (*Aphanocapsa*, *Nostoc*).

In the filamentous *Cyanophyceae* both the trichome and the individual segments composing it must be taken into account. In certain *Oscillatoriaceae* (*Spirulina*, *Oscillatoria* spp.) it is not easy to determine

whether the plasmic body is a trichome or a very elongated archiplast, since the transverse membranes are feebly developed or absent. It has, in fact, been suggested (13) that the single archiplast of the *Chroococcaceae* is homologous with the trichome of the *Oscillatoriaceae*.

Amongst important characters affecting the form of the trichome and varying from species to species (Fig. 2) are the constriction of the surface at the transverse walls, the forms of the apices of the trichomes, explained by Brand (10) as due to different degrees of atrophy, and the degree of curvature of the axis of the trichome. This latter character reaches its maximum in *Spirulina* (incl. *Arthrospira*), giving the trichome its regular spiral form, but curvature also occurs in other genera to a very varying extent among the different species.

True branching of the trichome must be regarded as producing the most extreme modifications of the form of the individual trichome. False branching of the trichome, on the other hand, must be classed as the production of a new trichome since it involves complete rupture in the transverse plane.

#### DEGREE OF DEVELOPMENT OF ENVELOPE.

The average thickness of the mucilage layer varies very greatly in different species and serves as an important systematic distinction. As this envelope is undoubtedly a mixture of chemical substances, another factor, namely the relative proportions of the various constituents, should also be taken into account. In systematic work this is at present chiefly recognised in differences of consistency. Apparently the proportion of water in the envelopes is particularly variable. All stages exist from a membrane of consistency equal to that of a cellulose wall to that in which the material is so dispersed as to diffuse freely into the surrounding medium.

Certain substances which are present in the envelopes of some species are absent in others. This is most clearly exemplified by the pigmented envelopes which occur more particularly in the *Chroococcaceae*, *Scytonemaceae* and *Stigonemaceae*. In the second of these families the genera which have several trichomes in the sheath are classified thus by West (38):

A. Sheaths containing schizophycin:

*Schizothrix*, *Dasygloea*.

B. Sheaths not containing schizophycin:

*Microcoleus*.

Tilden's classification (36) is in essentials the same.

In the genus *Lyngbya* the microchemical reaction with zinc chloridide differs along with other characters constituting specific differences (23).

Differences in optical properties of the different layers of the envelope are sometimes present. Variation in such stratification, the development of which was dealt with elsewhere (13), indicates a difference in chemical constitution between the species or forms concerned. A distinction was made between periodic metabolic changes in the membrane directly due to the process of cell division and those which could not be related to the division periodicity (13).

A very constant distinction between certain groups of filamentous species is found in the character of the sheath termination. In one section of the *Oscillatoriaceae*, for instance, the sheaths are open at the apex. In the other their ends have a tapering form, owing to their contraction after the escape of the hormogone (23). This evidently implies a difference in the material of which the sheath is composed.

The heterocysts are specialised archiplasts, the presence of which in certain species and not in others constitutes a discontinuity in the natural system. Their formation involves a change in the cell contents as well as a modification in thickness and chemical composition of the cell wall (39). The development of a calyptra of varying type is a further example of a specific character owing its origin to differentiation in thickness in the cell wall in different parts of the filament.

We have thus grouped under one heading modifications of the sheath and of the cell membrane itself, in spite of the very considerable microchemical differentiation which is known to exist between these organs in the *Oscillatoriaceae* (39). It is very doubtful whether this differentiation exists in unicellular forms, where the envelope must clearly be conceived as an excretory product not fundamentally different from the secretory products occurring as cell contents (13).

#### NUMBER OF ARCHIPLASTS OR TRICHOMES WITHIN THE ENVELOPE.

##### FALSE BRANCHING.

The arrangement of archiplasts or trichomes within the envelope is largely the outcome of their mode of division. This has been dealt with above. The actual number, however, will depend in part on the nature of the sheath, but also upon the rate of multiplication of the elements. Variations in the latter factors must therefore be considered as causes of systematic differences.

False branching (*Scytonemaceae*) is due to the rapid multiplication of the trichomes without a concurrent change in the nature of the sheath,

which, however, normally becomes ruptured to allow egress of the new element. False branches may afterwards escape and become new plants (5).

#### NUMBER OF PARTIAL COLONIES IN THE COMPOUND COLONY.

Just as the elements of the first order, *i.e.* archiplasts in the *Chroococcales* and filaments in the *Nostocales* and *Oscillatoriales* show varying phases of incomplete separation due to the possession of a common envelope, so the elements of the second order thus built up (simple colonies) may, in their turn, show grouping as partial colonies in colonies of higher orders (compound colonies). The existence of the "plant mass" as described in diagnoses of many species is due to this fact. Its superficial characters depend on a number of the factors enumerated in the foregoing.

#### COMMON MECHANISM OF SPECIFIC DIFFERENCES.

The series of taxonomic characters differentiating the various members of the *Cyanophyceae* have now been reviewed. The classes of characters mentioned include all those in general use among systematists. A striking feature is the predominance of simple quantitative differences, not only between species, but even between genera and higher systematic units. To this must be added the fact, familiar to taxonomists, that individuals transitional between species are exceedingly common among the *Cyanophyceae*. This was exemplified by some recent work by the present author in which the variation in collections of *Cyanophyceae* was particularly studied (14, 15). The determination of many species of these organisms is not complete without an observation of the range of variation exhibited by individuals in the same habitat.

It is obvious that many of the systematic characters reviewed above are due to simple differences of metabolic rate. Of course, all the characters of the higher organisms can be reduced to growth variations, but in these the fact is often obscured by the discontinuity between species. In many *Cyanophyceae* the differences between species are in one character only.

We have, at present, no accurate knowledge of the variations in metabolism in the *Cyanophyceae*. In the Bacteria such variations are of great systematic value since they have been proved to be constant in culture. The work of Gurney-Dixon (24) shows how rare mutations in metabolism are in this group. The most recent system of bacterial classification, adopted by the Society of American Bacteriologists and founded

on the general results of numerous workers(41), gives the character of the metabolic processes an important place in the evaluation of genetic affinity. The characters of the metabolic processes have also been recognised as criteria of relationship among the higher plants(3). Reference may also be made to the fact that serological characters are found in the main to confirm the morphological characters as indications of affinity. Nuttall and his associates(30) proved this for numerous animals, mostly vertebrates. Other recent workers have added data from invertebrates and plants.

Although in the *Thallophyta* chemical differences between species have scarcely been studied, the main classes appear to be distinguished by differences in metabolism. The *Cyanophyceae* as a group are characterised by the glycogen<sup>1</sup> content of their cells. In other groups of coloured Protista the chief metabolic product is starch (*Isokontae*, *Akontae*, *Cryptomonadales*), oil (*Heterokontae*), paramylon (*Englenales*), or leucosin (*Chrysomonadales*). Glycogen is, however, found in the mycelium of many fungi(19).

If the systematic differences in *Cyanophyceae* are of the nature of variations in metabolism, it should be possible by cultural experiments to produce variants from a given species having the characters of a related species or genus. Such experiments will be described below. Further, it is known that individuals of a given species growing under different conditions in nature show differences precisely similar to those heritable differences found among members of different species growing in the same habitat.

#### CONSTANCY OF SPECIES IN *CYANOPHYCEAE*.

Whilst profound changes in the morphology of these algae actually do occur under certain conditions, there is every reason for supposing constancy of specific type is maintained under natural conditions as in other groups of organisms.

Many of the *Cyanophyceae* grow normally in damp terrestrial situations and these forms can be grown in culture on porous porcelain, the pores of which are filled with the culture medium by capillarity. Knop's solution, with or without a trace of peptone in addition, is suitable; Molisch's solution, adopted by Brunnthaler(11), may also be used. Various

<sup>1</sup> Glycogen is identical in molecular composition with starch and not merely an isomer of that substance as formerly supposed. The very obvious difference of the two substances in colour reaction with iodine, however significant, does not necessarily indicate a metabolic difference comparable with that distinguishing the various classes of Bacteria.

species of unicellular and filamentous forms have been grown in this way for several months without any divergence from the characteristic specific form. Aquatic forms have also been kept in culture solutions and are found to grow much more readily than the majority of Green Algae. *Tolypothrix lanata* (Desv.) Wartmann, for instance, was kept for over two years without change of form, even such details as grouping of heterocysts, thickness of sheath and mode of branching remaining constant. *Phormidium tenue* (Meneg.) Gom. was also kept without change for about a year. It is further possible to grow species under conditions, which are unlike the natural environment, without change of form, although as will be seen later, environmental changes of certain kinds may actually induce change. Typical aquatic species of *Oscillatoria* and *Phormidium* were grown on porous plates for several weeks without change of form. A remarkable instance of conservation of form was seen in some cultures of *Tolypothrix lanata*. This species, besides being grown in diluted Knop's solution, was kept in parallel cultures in the same solution to which, however, varying amounts (1, 2.5, 5, 10 per cent.) of sodium chloride were added. The material was obtained by cutting up a single colony. Throughout their growth all the specimens retained their specific characters without change so that it would be impossible to distinguish those grown in 10 per cent. NaCl from those in normal culture.

Changes induced experimentally are also found to persist as long as the cultural conditions are constant, but the following experiment shows that the organism does not lose its specific potentialities. Some colonies of *Microcystis aeruginosa* Kuetz. (Fig. 3 C) were placed in a culture dish containing 2 per cent. Knop's solution and allowed to stand in an exposed, well-lighted position for about two weeks. After that time they were found to have retained their original form and were in an actively multiplying condition. From this culture typical colonies were isolated by means of a small pipette and transferred to sterile porous porcelain blocks, after microscopic examination of the contents of the pipette to ensure that no foreign Algae were admixed. The porcelain blocks were saturated with sterile 2 per cent. Knop's solution and the colonies were able to grow on them. In this state (Fig. 3 B) the organism resembles some of the terrestrial species of *Aphanocapsa*, except that the gas vacuoles appear to be retained. Large quantities of glycogen are present in the cells as shown by their very intense brownish red coloration with iodine. If a loopful of this mucilaginous stage was transferred to 2 per cent. Knop's solution, the typical colony form finally reappeared. At

first the colonies were not clathrate or lobed, but simple in outline and might be mistaken for those of *Microcystis flos-aquae* Kuetz. But after continued growth and reproduction by division of the first formed colonies they reverted to the typical clathrate form. The process took several weeks.

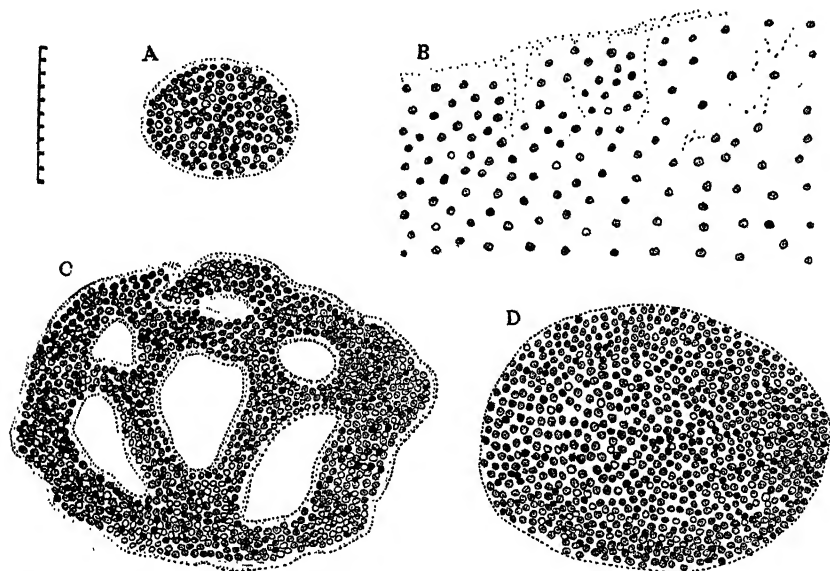


Fig. 3. Developmental stages of *Microcystis*. A, B and C. *Microcystis aeruginosa*. D. *Microcystis flos-aquae*. A. Young colony. B. Colony grown on porous porcelain. C and D. Normal adult colonies. Each scale division measures 10 microns.

Other instances might be given of the constancy of specific characters in *Cyanophyceae*. The case cited shows that a character (colony form) which is undoubtedly very susceptible to environmental influences may definitely persist in inheritance. All the characters referred to in the next section, as being modified by experimental changes in the medium, have been found to remain constant in culture if the organism is kept under normal conditions.

#### EXPERIMENTAL PRODUCTION OF VARIANTS.

The forms of *Microcystis aeruginosa* Kuetz. produced by culture on porous porcelain have already been referred to. In these the plant loses its definite colonial plankton form and becomes an ill-defined creeping stratum similar to the normal form of many rock growing species of *Aphanocapsa*. Plankton species of *Microcystis*, such as *Microcystis aeru-*

*ginosa* and *Microcystis flos-aquae*, when grown in adverse conditions of culture in liquid media have also been kept in an indefinite benthic form, similar to species of *Aphanocapsa*, for some weeks. The readiness with which *Microcystis* is converted into these *Aphanocapsa* forms supports the view, already put forward by the present author on other grounds, that some members of the latter genus are closely related to, and probably cogenetic with *Microcystis* (14). Elenkin, in his recent classification of the *Chroococcaceae* (18), actually merges these genera in one. But this author, as all other recent authorities, maintains the *Aphanocapsa* forms occurring in nature as distinct species. The old idea that the naturally occurring forms of *Cyanophyceae* are numerous polymorphs of a few main types, receives no support from modern systematists.

Modifications of the envelope may also be induced by alterations in the environmental conditions in many filamentous species. My own experiments dealt mostly with members of the *Oscillatoriaceae*, a family in which the generic characters are based on the nature of the envelope. Yet the form of the sheath is probably more affected by the influence of the medium than the characters of the enclosed trichome, a fact which must have considerable bearing on the classification of the group.

In pure cultures of *Oscillatoria* on sterile soil which had been allowed to undergo periodic desiccation for some months the sheath became more membranous than in forms grown in suspension in Knop's solution for the same period.

In *Phormidium tenue* (Meneg.) Gom. the effect of light was studied. Portions of a single colony were grown in Molisch's solution, diluted with an equal quantity of distilled water, in light and darkness for several months<sup>1</sup>. A very pronounced difference between the cultures resulted. In the colonies grown in the light the very compact mucilage was obvious and the trichomes were very closely entangled (Fig. 4 A). In those grown in darkness mucilage was not visible and the trichomes were much more loosely intertwined, so that the meshes of the colony were of four to five times greater area than in the specimens grown in the light (Fig. 4 B).

When cultures of *Phormidium tenue* are exposed to bright light the mucilage becomes brownish in colour. This was not noticed in the above-mentioned cultures, which were not exposed to full daylight, but in another series of cultures made up in Molisch's solution diluted with 50 per cent. water and derived from a single piece of the colony showing

<sup>1</sup> The *Cyanophyceae* retain their colour when grown in darkness. Some species of *Lyngbya* are found naturally in dark caves.



no sheath coloration. Some<sup>1</sup> of these cultures were grown for about a month, fully exposed to sunlight. Others were grown for the same period in a shaded situation and in the dark. Only in the well-lighted series was any coloration of the sheath noticeable and in these it was definite.

Another character was noted in these well-lighted cultures and was not seen in any of the others. This was the presence of spiral filaments (Fig. 4 C). Many of the filaments in each of the plants exposed to the

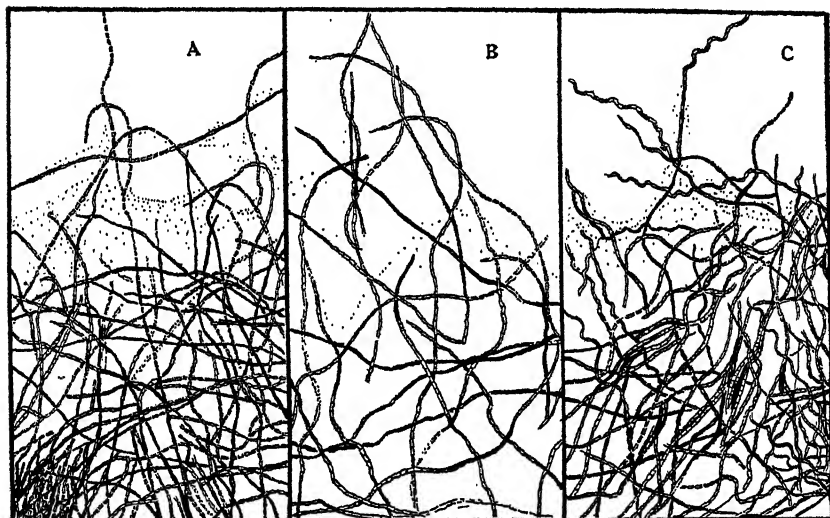


Fig. 4. *Phormidium tenue*. Portions of colonies. A. Grown in daylight in the shade. B. In darkness. C. In daylight, fully exposed to the sun. From preparations stained with methylene blue.

light consisted of definite spiral turns between  $36\text{--}40\mu$  in length and  $12\text{--}15\mu$  in width. Besides these definite spirals looser and more irregular forms were present, as well as practically straight or simply bent filaments, as in the cultures which were not grown in full daylight. The definite spirals are similar to the normal forms of certain species of *Lyngbya*. In one of the strongly illuminated cultures definite spirals of regular form, but wound very closely, were met with. These have a trichome form characteristic of the genus *Spirulina* (incl. *Arthrospira*), although of course the dense colony is totally different.

The addition of peptone to cultures of *Phormidium tenue* in mineral salts is favourable to the development of a firm colonial mucilage. Culture solutions consisting of 50 per cent. Molisch's solution and 50 per cent.

<sup>1</sup> About six culture tubes were studied in each series of this and the other experiments described.

of 0.5, 1.0 and 2.0 per cent. peptone solution (*i.e.* containing 0.25, 0.5 and 1.0 per cent. peptone respectively) gave increasingly distinct colonial mucilage, the individual sheaths of the filaments also becoming obvious in the two higher concentrations (Fig. 5 B). In control cultures without or with only a trace of peptone the mucilage was diffuent and showed no distinct outline in unstained specimens (Fig. 5 A). The density of the

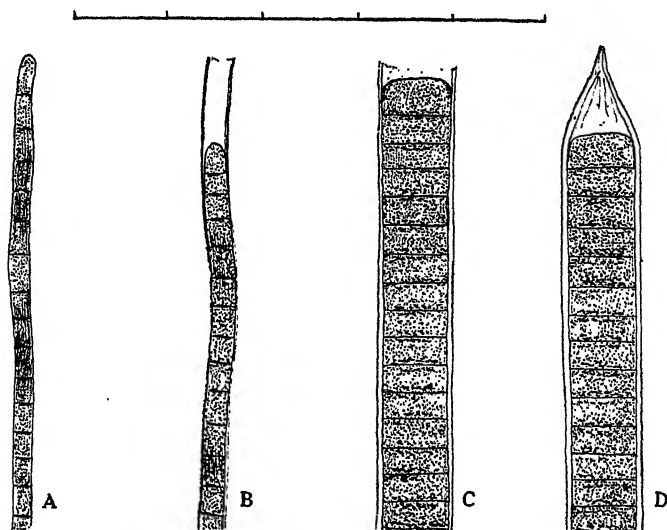


Fig. 5. A. *Phormidium tenue*. Portion of filament grown in media without peptone. B. The same grown in media containing 1 per cent. peptone. C. *Lyngbya nigra* portion of filament normal. D. The same grown in the presence of 0.5 per cent. fructose. Each scale division measures 10 microns.

intertwining of the filaments was not observed to vary in these cultures as in the light and darkness series. In nature it is possible that some of the effects of peptone may sometimes be produced by other organic substances which were not present in the cultures. But ordinarily the mucous condition of the envelope predominates in nature. The forms with firm mucous sheath of the stronger peptone cultures would certainly be classed as a distinct species. The presence of very definite individual sheaths closely approaches the typical condition of *Lyngbya*.

Variations of a definite kind have been observed in cultures of *Lyngbya nigra* Ag. In the original culture of this species the sheaths were open at their apices (Fig. 5 C) except in very rare instances. This open character of the sheath is one of the most constant distinctions of the tribe *Lyngbyeae* (*e.g.* *Lyngbya*, *Symploca*, *Plectonema*) from the tribe

*Vaginarieae* (e.g. *Schizothrix*, *Dasygloea*, *Microcoleus*) and it is correlated with several other distinctions (23). Yet in pure cultures of *Lyngbya nigra* containing 0.5 per cent. of fructose practically all the filaments terminated in closed tapering ends (Fig. 5 D), exactly as described by Gomont for the *Vaginarieae* (23). Glucose and sucrose do not appear to produce the same effect. These results were obtained after three weeks' active growth.

It is not probable that the changed form was a direct stimulating effect of the fructose since traces of acetone appear to have the same effect. Closed tapering ends appear to be commoner in cultures which are not in a healthy condition and the character is probably pathological for *Lyngbya*. Bouilhac (9) has shown that glucose and sucrose, but not fructose, can be assimilated by *Nostoc*. The same may be true of *Lyngbya*. But that other factors are involved is shown by the fact that cultures without any carbohydrate show normal non-tapering ends. Possibly sugars have a depressing effect on photosynthesis.

Colour changes and variations in the development of the transverse walls, and with regard to the presence or absence of transverse granulations, all of which are of systematic importance in the genus *Lyngbya*, occur in cultures of *Lyngbya nigra*. The determining factors were not studied. The same applies to certain variations in the development of the transverse walls which may practically disappear in cultures in a liquid medium (Molisch's solution). This was observed in the terrestrial *Cylindrospermum licheniforme* (Bory) Kuetz.

Among unicellular forms analogous cell changes have been observed. It is generally assumed that cell size is affected by growth factors. In *Gloeocapsa*, however, a single species may exhibit variants which differ in size from the normal type to the extent of three or four diameters. In a culture of an unidentified species of *Gloeocapsa* (Fig. 6 A) in a small vessel in about 2 c.c. of Knop's solution the cell gradually decreased in size until at the end of six months (Fig. 6 B) they were about a fourth of their former size. The normal envelope was retained and the colonies did not change in size.

The number of the cell generations in each colony had thus increased and the new colonies therefore differed in a character which is of systematic value in the genus *Gloeocapsa* and which remained constant in parallel culture tubes containing a more abundant supply of culture fluid (more than 10 c.c.).

Wille (40) observed the production of a similar small-celled form in *Gloeocapsa crepidinum* Thur. accompanied with a modification of the membrane, which loses its characteristic form and takes on an amor-

phous character. The whole plant then resembles the forms described as *Aphanocapsa marina* Hansg. which Wille therefore considers to be a phase of *Gloeocapsa crepidinum*. Wille states that it appears as if continual humidity favours the development of the *Aphanocapsa* phase, whilst regularly recurring and long desiccation favours normal division. Possibly the effect of desiccation has played a part in the phyletic

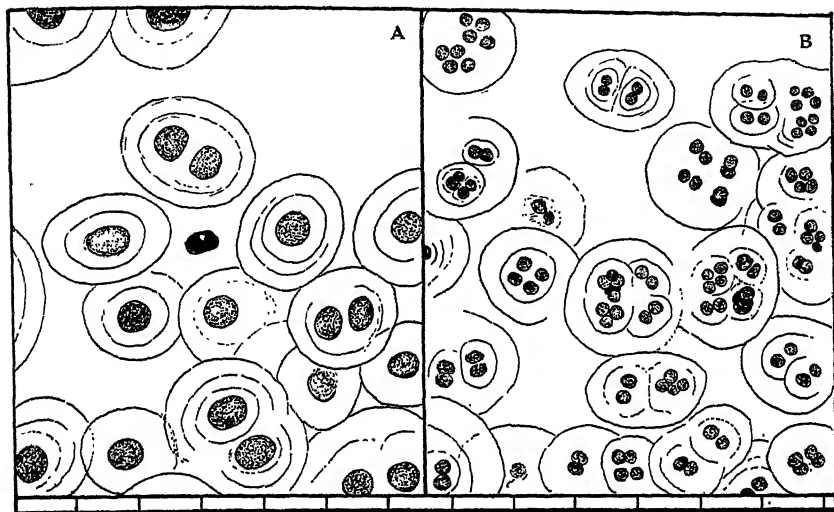


Fig. 6. *Gloeocapsa* sp. A. Normal colonies. B. Colonies with reduced cells. Each scale division measures 10 microns.

development of the genus *Gloeocapsa*. The characters of the latter genus cannot be regarded as merely due to effects of the dryness on the ontogenetic development of certain organisms, for the experiments mentioned above, as well as others performed by the present author, show that the characteristic envelope and mode of division may persist for numerous generations in an aquatic environment.

#### OCCURRENCE OF VARIATION IN GENERAL.

We have now considered two distinct types of variation, namely the inherited variation which constitutes the systematic differences and the acquired variation resulting from changes in environmental factors. Both of these appear as changes in metabolic processes. Other kinds of variation may also be distinguished. The stages through which a species passes in its ontogeny, for example, may be described as age variants of that species. Three examples will be cited from as many different

families, but it would be possible to add many other illustrations of the special feature of age variation referred to below.

The colonies of the *Chroococcaceae* never attain a high degree of complexity, but some of the larger species, belonging to the genera *Microcystis* and *Coelosphaerium*, commonly exhibit a clathrate structure. This is characteristic of *Microcystis aeruginosa* Kuetz. (= *Clathrocysts* Henfr.) for instance. Nevertheless, normal young colonies (Fig. 3 A) may not possess this important feature. It is certain that in the normal life-cycle of species of these genera age variations occur which closely resemble systematic differences. Wesenburg-Lund<sup>(37)</sup> has described the variations occurring in the yearly cycle of generations and finds them to be of the same nature. This author, in fact, considers all the plankton forms of *Microcystis* to belong to one species. Inasmuch as we have already shown a strong tendency to constancy of form even in the colonies of this genus this conclusion cannot be accepted if it implies that genetically distinct lines do not exist. On the other hand, the similarities between the forms described are so close as to warrant the application of the term microspecies to them.

Again in the individual life-cycle of the *Oscillatoriaceae* the various stages which are known to belong to a single species are sufficiently distinct to be placed in different species if their genetic connection were unknown. In fact, the differentiation of the life-cycles of the higher members of the group is so great that the organism passes through a series of different generic types. The hormogone is the young phase in all species of the family (Fig. 7 A) and it serves for distribution of the plants. In the genus *Oscillatoria* as is aptly expressed by Gomont<sup>(23)</sup> the hormogonial state is permanent. In certain circumstances one sees them secrete sheaths, but these are too tenuous to unite them to form a colony.

In *Lyngbya* and allied genera the hormogonial filaments develop tubular sheaths around them, forming the adult plants from the trichomes of which by division the hormogones are produced. Finally, in the genera *Microcoleus*, *Hydrocoleus* and *Schizothrix* the *Lyngbya* condition (Fig. 7 B) is superseded by the division of the trichomes within the sheath so that an outstanding systematic character of this group, namely the inclusion of several trichomes within the sheath (Fig. 7 C) is developed. The common sheath which enclosed them in *Microcoleus* takes on a considerable thickness and often acquires a lamellose structure. Some species, such as *Microcoleus versicolor* Thur. have individual sheaths around each trichome, as well as the common sheath surrounding the

whole mass. Bornet and Thuret(4) first observed hormogone formation in such species of *Microcoleus* and recorded that division to form hormogones always takes place within the common sheath.

Finally, the development of the well-known globular colonies of *Gloiothrichia* from the spores, as fully described by Wesenbourg-Lund(37) may be cited. The spores grow into filaments of the *Oscillatoria* type.

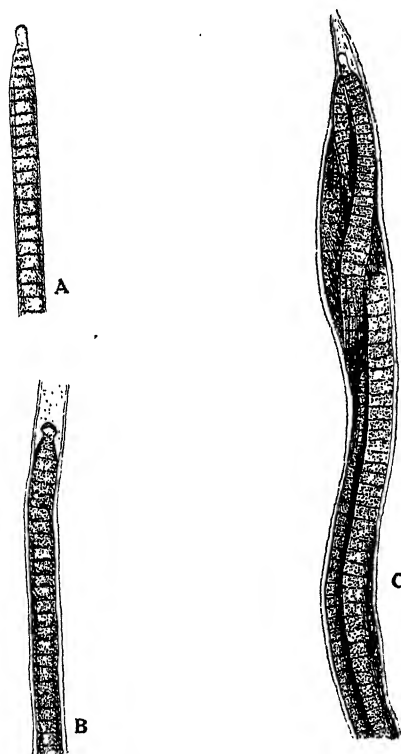


Fig. 7. Developmental stages of *Hydrocoleus Brebissonii*. A. *Oscillatoria* stage (hormogone). B. *Lyngbya*-stage. C. Adult.

The straight single filaments then become associated in tufts which Wesenbourg-Lund speaks of as the *Aphanizomenon* stage<sup>1</sup>. It is, according to Wesenbourg-Lund, by the bending of the filaments of the latter, the development of two heterocysts at the middle, and subsequent breaking of the filament between the heterocysts, and development of the fine tapering apices, that the colony takes on its adult form. Thus, in colony-form, the more juvenile age variants of *Gloiothrichia* have all

<sup>1</sup> For figure see Wesenbourg-Lund (37).

the characters of the adult colonies of plants of relatively remote systematic affinity. The parallelism between the ontogenetic and the taxonomic series is, in the characters cited, as in others that could be mentioned, not a theory, but an observed fact.

Morphological differentiation of an individual involves variation amongst the homologous parts of the individual, another very extensive class of phenomena which can only be referred to here in its direct bearing on the species question. In the more highly differentiated forms it is clear that differentiations, which are observed to be identical in character with the variations between species, occur between different parts of the same plant. We need only refer to the tapering form of the filament in the *Rivulariaceae* and the variations in the thickness of the sheath in different parts of the filament in certain *Scytonemaceae*. The mechanism of morphological differentiation is now generally conceived along dynamical lines. The theory of Child<sup>(12)</sup> is specially convincing when applied to the structure of the Algae, particularly the *Cyanophyceae*, since here the existence of simple metabolic gradients is implied by simple gradients of structure. Examples may be found in the filaments of *Rivulariaceae* and some *Scytonemaceae* and in the reproductive bodies of *Chamaesiphonaceae*. But other forms of structure, implying other forms of metabolic gradation must be taken into account. For example, in simple unicells like *Chroococcus* the structure implies a gradient from the centre of the cells outwards (central body, peripheral plasma, envelope).

Whatever theory is accepted, the phenomena of morphological differentiation, imply a division of labour in metabolism.

Besides normal morphological differentiation varying degrees of abnormal changes are met with. Some of the latter were described in a previous publication<sup>(13)</sup>, since they are sufficiently common to have a bearing on classification. In the colonial *Chroococcaceae*, for example, extreme variations in distance apart of the cells may occur in one and the same colony. On the basis of this observation it was claimed that the character in question had small taxonomic value. This no doubt is true; the character in question is relatively worthless in classification, but it is also evident that as in cultures the character may be inherited for long periods, it is not altogether without systematic value. All observations tend to show that all characters, even supposed pathological ones like the apex form of *Oscillatoria*<sup>(10)</sup> may have some systematic significance.

The filamentous forms, as well as the *Chroococcaceae*, which have been illustrated elsewhere<sup>(13)</sup>, not infrequently show abnormalities, so that even in different parts of one and the same plant the structure may differ

in the same way as amongst normal representations of different species. The following are clear examples that have recently come under my observation; a full account of the phenomena, however, would necessitate a monographic description of the whole group.

In the genus *Lyngbya* the length of the segments and the distinctness of the cell walls are important distinguishing characters. An examination of a pure culture of *Lyngbya nigra* Ag. shows that on the whole these characters show remarkable constancy. Thus 162 hormogones of this species were examined. One hundred and fifteen showed little or no variation in the length of their segments which measured in fact  $3-4\mu$ . Further, the transverse walls in all these were apparently equally developed. In seven hormogones, on the other hand, the structure was modified at certain points by the suppression of one or two transverse walls, resulting in segments of twice or more than twice the normal length.

It is well known that the dimensions of the heterocysts are important diagnostic characters in many genera which possess them. In *Scytonema* they are also, on the average, different in different species, but here the individual variation may approach the specific variation. In *Scytonema tolypotrichoides* Kuetz. the following measurements of the length of 22 heterocysts of a single plant were made. The width in all cases was practically constant at  $9\mu$ .

Length of heterocyst:

10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29

No. of heterocysts:

3, — 2, 2, 2, — 2, 2, 3, 1, 1, 1, — 1, — 1, — 1, — 1

On the other hand, in many *Nostocaceae* the heterocyst may not vary within measurable limits in a single plant, yet there is no doubt that this organ is homologous in the two families.

The generic distinction between *Scytonema* and *Tolypothrix* is well known to be difficult to determine in some specimens. Whilst the majority of plants either show predominantly the false branches arising in the interval between two heterocysts (*Scytonema*) or at the heterocyst itself (*Tolypothrix*) it is not uncommon to find both types of branching well developed in a single plant. The different sheath types, distinctive of *Petalonema* and *Scytonema*, have also been observed on a single plant (Fig. 8 A). Although the frequency of this occurrence has not been studied, it is certain that many plants constantly show one type of sheath only. The genus *Hypheothrix* shows similar phenomena. In this genus the sheath may contain one or more trichomes, both conditions



being commonly met with side by side in some species. The species *Hypheothrix coriacea* Kuetz. is described as having cells of length greater than the diameter. In many filaments this is found to be so without exception. But the author has examined filaments in which amongst normal segments there also occur isolated ones that are distinctly of lesser length than the diameter (Fig. 8 B). The allied species *Hypheothrix*

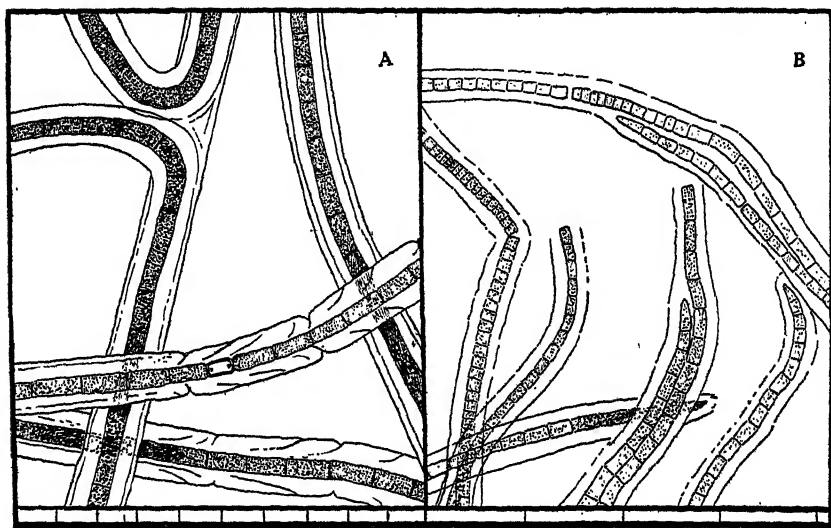


Fig. 8. A. Abnormal filaments of *Scytonema* sp. showing *Petalonema* characters.  
B. *Hypheothrix coriacea* showing variation in size of cells.

*Lenormandiana* (Gom.) Forti and *Hypheothrix vulpina* Kuetz. are characterised by cells constantly shorter than broad.

A final example may be given from the genus *Spirulina*. In this genus the distance apart of the coils of the spiral filament is generally constant. In pure cultures there is frequently no measurable difference. Yet abnormalities are occasionally met with in which different parts of a single filament may show marked variation in this character. Setchell and Gardiner(35), for instance, figure a specimen showing the tightly coiled character of *Spirulina subsala* Oersted and the loose coils of *Spirulina major* Kuetz. on a single trichome.

In general the extreme variants of a series of individuals of a given species shows some of the characters of an allied species, so that frequently it is necessary to examine a number of individuals to establish its identity. The existence of transitional forms between species, a phenomenon familiar to every systematic biologist, is thus specially striking in the *Cyanophyceae*.

## RELATIVE VALUES OF CHARACTERS IN CLASSIFICATION.

Theoretically, it should be possible to arrange all the characters of a group in order of their value in classification. Practically, it is very difficult to make such a definite arrangement, even when dealing with a group of such simple organisms as the *Cyanophyceae*. Moreover, the characters that are relatively constant in one genus may be less constant in another and so of less systematic value. Much might be done by a review of the known facts of variation and certain conclusions have already been drawn in an earlier publication<sup>(13)</sup> with regard to the *Chroococcaceae*.

In the present work the cases studied have been too isolated to draw detailed conclusions as to the systematic grouping of the families and genera, the main object being an investigation of the principles underlying the different kinds of variation. Consequently special controlled cases have been described, rather than a complete collection of the known facts regarding variation. Nevertheless the evidence given in these pages shows that in both unicellular and filamentous forms the external form of the colony is extremely susceptible to the environment. Elenkin's<sup>(17)</sup> stress on the external characters in classification is not in harmony with experimental work. The degree of development of the envelope and the pigmentation of the archiplast may be instanced as very difficult characters to apply in taxonomy. On the other hand, the mode of cell-division and structure of the archiplast<sup>1</sup> and trichome are far more reliable. It is not claimed, however, that similarity of mode of division alone can be used in bringing together forms of close systematic affinity. A satisfactory classification must rest upon a review of all the characters, together with a proper evaluation of their hereditary constancy.

## RELATION OF CHARACTERS TO CELL STRUCTURE.

The general nature of the changes involved in specific differentiation, which can be inferred from the review of the systematic characters of the *Cyanophyceae* given in previous pages, has already been mentioned. Obvious saltations are rare. In actual studies of variation of individual species so many intermediate forms are frequently found that the systematists may differ considerably as to the limits of what may be conveniently regarded as a species, *i.e.* a linneon in the sense of Lotsy<sup>(25)</sup>. In this respect the *Cyanophyceae* resemble many other groups of Protista,

<sup>1</sup> Gardner (21) states that change of habit does not produce any marked change in cytological characters.

especially certain Fungi. Amongst animals, the corals offer many excellent examples. On the other hand, as Bateson has demonstrated (2), discontinuous variation is a remarkably widespread phenomenon. Even in the *Isokontae* excellent examples of discontinuous variation are met with in such characters as the position of the eye-spot and the number of flagella in the swarmer (16). In the *Isokontae* there is a remarkable parallel, in general morphology, with the *Cyanophyceae*, a fact that will be referred to later. But their cytology and mode of reproduction are so different that there can be no question of relationship between the two groups.

It is not impossible that a gradual differentiation of metabolic rate might lead to discontinuous variation. The physiology of metabolism is not without examples of periodic phenomena. Even in the secretion of membrane periodic structure may owe its origin to purely physical factors, such as are concerned in the production of the Liesegang rings (13) rather than in any periodicity in the secretion process itself.

Apart from the metabolic aspect of the phenomenon there is, however, the cytological one. Among most *Isokontae* definite sexual processes occur. In a previous publication (16) the possibility of a correlation of the occurrence of sex with the presence of discontinuous variations in the *Isokontae* was remarked. It is therefore noteworthy that definite discontinuities in variation have not been observed between closely allied species or between different phases of a single species in the *Cyanophyceae*.

Another important difference between the two groups, in view of the correlation between cytological and genetical phenomena, now so well established in many animals and plants, lies in the very different cell structure of the *Cyanophyceae* and *Isokontae*. Various views have been propounded with regard to the central region of the archiplasts of the former, but all authors are now agreed that it differs very considerably from the typical nucleus of higher animals and plants. The *Isokontae*, on the other hand, in the cases investigated (see (27), (39)), show the presence of chromosomes and the process of karyokinesis.

West (39) suggested that the relatively undifferentiated character of the Cyanophycean cell might be connected with the absence of sexuality in this group. For absence of sexuality is characteristic of the *Cyanophyceae*. It is true that both Phillips (34) and Borzi (6) have observed a fusion of adjacent archiplasts in a filament at spore formation. According to Phillips as many as four archiplasts may go to form a single spore. In view of the facts regarding individual variation in the development of

septa recorded above, and in the specific variation of these septa, especially within the genus *Spirulina* (incl. *Arthrospira*) the suppression of septa during ontogeny cannot be looked upon as a purely sexual process. Free gametes have certainly not been proved to exist in the *Cyanophyceae*.

According to the recent theory of the evolution of sexual reproduction, due to Franz(20), the origin of fertilization is attributed to the mechanism of karyokinesis. When the latter process was first developed, occasional abnormalities resulted in reduction divisions by disturbances of the normal process. Fertilization, at first a rare and abnormal process, would restore the normal chromosome number and would thus be favoured in natural selection. The arguments in favour of this theory cannot be cited here, but it is in harmony with the apparent connection between archaic cell structure and asexuality in the *Cyanophyceae*, a fact not mentioned by its author.

In addition to the visible peculiarities of structure of the Cyanophycean archiplast and trichome, the protoplasm appears to possess other characters in which it differs from that of the majority of plant and animal groups. Like other groups of Protista the *Cyanophyceae* have their own peculiar microchemical characters. Thus they have a distinctive membrane composition and a special pigmentation. The presence of glycogen as the chief metabolic product has already been referred to. But these distinctions are obviously of the same order as existing between the *Isokontae* and *Heterokontae* or between the various classes of Bacteria for instance.

A more fundamental peculiarity appears to reside in the physiological properties of the archiplast however. It is well known that the *Cyanophyceae* are extremely resistant to high temperatures; they can, moreover, carry on very active growth at temperatures which are fatal to the majority of organisms. They constitute the chief vegetation of hot springs. To this may be added their resistance to high osmotic pressure, desiccation and absence of light which have been well illustrated in some of the experiments recorded in previous pages. In these, and other features, the *Cyanophyceae* are only paralleled by certain Bacteria and Fungi(28). The resistivity cannot depend to any great degree on the mucilage envelopes of the plants. Not only is it not shown by many species with feebly developed mucilage but many Algae belonging to groups other than the *Cyanophyceae* possess equally well developed envelopes of similar physical constitution, without showing the peculiar resistive power of members of that group. The subject has been dealt

with in detail by Mereschkowsky (28) who ascribes a special constitution to the protoplasm of *Cyanophyceae*, Bacteria and Fungi. The author speaks of this special form of protoplasm as mycoplasma<sup>1</sup> and separates the groups that possess it from all other classes of organisms.

The peculiar and probably archaic cytological organisation of the *Cyanophyceae* has been correlated with the nature of the systematic differences within the group. In organisms at the other end of the scale of classification it has been possible to interpret the systematic distinctions as due to the presence or absence of Mendelian unit factors (22). MacBride (26), however, points out that the majority of mutant dominant Mendelian characters are quite unlike those distinguishing species. According to Gates (22) characters are of two kinds, organismal and karyogenetic. In view of the similarity of the systematic differences with those produced by the environment, the *Cyanophyceae* would have characters of Gates' former type only. This, then, is in harmony with the nature of the cytological structure of the group. This conclusion is not affected by the degree of variation within a pure line, a question which cannot be dealt with here. Further, that clones may exist just as definitely in the *Cyanophyceae* as in other groups is by no means affected by their cytology. The bacteria have a still simpler cell structure but definite pure lines are better known in these organisms than in many other plants and animals which show typical karyokinesis.

#### HOMOPLASY OF CYANOPHYCEAE WITH OTHER GROUPS.

It is remarkable that the great difference between the *Isokontae* and *Cyanophyceae* in cytological and protoplasmic organisation is not accompanied by great differences in somatic structure between the two groups. Homoplasy between certain *Cyanophyceae* and *Isokontae* is extremely well illustrated, but the facts have hitherto scarcely been studied in spite of the frequent references which have been made in the literature to the convergence of form in *Isokontae* and *Heterokontae*. The fact that the two latter groups have only been clearly distinguished as the result of comparatively recent investigations on the zoospore phase indicates the great similarity which exists (see (32)). Further examples were added by such discoveries as those of *Pseudotetradron* (31) and *Characiopsis* (7), Heterokontan genera which possess vegetative stages similar in form to the Isokontean *Tetradron* and *Characium* respectively.

The homoplastic development of certain *Cyanophyceae* with members of the *Isokontae* is definite in a number of isolated cases. There is

<sup>1</sup> This must not be confused with the supposed mycoplasma of phytopathology.

not, however, the parallelism of the group as a whole which has been pointed out in other classes(33), except in the existence of unicellular and filamentous types. Clear instances of homoplasy are found in the following examples. *Gloeocapsa* is parallel with the Isokontan genus *Gloeocystis*, both having the same type of well-developed envelope, apparently in relation to their terrestrial mode of life. The majority of species of *Merismopedia* are unique in their colony form and cannot be mistaken for other Algae. Nevertheless, some members of this genus, of which the best example is *Merismopedia Gardneri* (Collins) Setchell (35), pass over, by their slightly less uniform method of division and larger colonies, to forms homoplastic with the genus *Prasiola*. *Dactylococcopsis* is similar to *Dactylococcus* and *Ankistrodesmus* among the green Algae. The genus *Tetrapedia* shows a cell form essentially of the type seen in Desmids, for although of simpler form than in the majority of the latter, it possesses the characteristic bifurcation of the cell into two lobes, the semicells. Among the attached unicells belonging to the *Chamaesiphonaceae* species having the same forms as *Characium* (*Isokontae*) and *Characiopsis* (*Heterokontae*) exist. *Coelosphaerium* has the colony form of *Eudorina* (*Isokontae*) although the mode of reproduction is entirely different. Superficially *Gomphosphaeria* is very similar in form to *Pandorina* (*Isokontae*) although the colony of the former is constructed in a manner unknown in the *Isokontae*, each cell having primitively a distinct stalk or specialised base. The encrusting species of *Chroococcus* with a comparatively thin envelope are similar in form to *Pleurococcus* (*Isokontae*), whilst the allied *Synechococcus* is homoplastic with *Stichococcus* (*Isokontae*). Amongst filamentous forms the fact that some species of *Lyngbya* are difficult to distinguish from *Hormidium* and *Gloeotila* (*Isokontae*) may be cited. The spiral form, characteristic of certain other plankton organisms, occurs in both *Lyngbya* and *Gloeotila*. The globular floating colonies of *Gloietrichia* and some species of *Scytonema* find a parallel in *Aegrogrophila* (*Isokontae*).

Homoplasy and allied phenomena of convergence are of course extremely widespread in many groups of animals and plants. Its significance in the *Cyanophyceae* in connection with the foregoing investigations is in showing that a cell structure of very different type to that of the majority of other groups of Algae is capable of existing along with a body organisation sometimes almost indistinguishable from that of these groups.

## SUMMARY AND GENERAL CONCLUSIONS.

The systematic characters of the *Cyanophyceae* can, in general, be reduced to differences in the degree of development of a few main types of structure. They can be visualised as graduated variations of a few metabolic processes. Continuous variation is often apparent in the natural system. The differences between closely related species rarely show that discontinuity which is apparent in many other groups of organisms. This does not preclude the existence of unit factors, although not having assumed such factors, it is found unnecessary to introduce them to account for the characters.

The nature of the systematic differences in the *Cyanophyceae* is correlated with the mode of reproduction by asexual means, with the peculiar cytological characters and probably with the special physiological properties of the protoplasm. The analogy of certain discontinuous variations in the *Isokontae* and *Akontae*(16) with those met with in the offspring of a heterozygote in Mendelian experiments has already been mentioned. Many of these *Isokontae* and *Akontae*, however, show a definite sexual process, and hybridisation or processes of an analogous nature may there play a part in their causation. The absence of normal sexuality, at least in all typical species, enables us to study the phylogenetic series without having to take account of the anastomoses due to crossing. The results must therefore be considered as contributing to that fundamental aspect of variation referred to by Lotsy in the introduction to his work(25) on the rôle of hybridisation in evolution, but not further dealt with there.

Most authorities will admit that direct environmental influences on the reproductive cells play a part in inducing this fundamental kind of variation. Two lines of evidence are offered in the preceding pages in support of this. Firstly, homoplasmy shows that similarity of form in cell and colony is not excluded by dissimilarity of intimate cytological structure and probably also by a very different physico-chemical constitution of the protoplasm. Secondly, variations which in their observed character and apparent growth mechanism differ in no way from those existing between species, may be produced in a single species by the action of various environmental factors. This latter fact has been shown by experimental methods. The conclusion may hold good that all characters might be produced by suitable alterations in the environment, but in many groups the fact, if true, is certainly obscured by re-combination of the characters due to the sexual method of reproduction, and possibly

by the fact that the stimulus necessary to evoke such characters cannot be reproduced experimentally.

The problem of the cause of that diversity of forms constituting the natural system of organisms is one that must always be studied in the light of the actual observed systematic differences. On the other hand, as it is a problem of protoplasmic differentiation, the study of responses during the individual life must necessarily throw light on the process. Only in the syntheses of these two points of view can the problem be adequately investigated. The chief importance of taxonomic research lies in its contribution to our knowledge of the actual results of the most fundamental of all biological processes. The lower organisms afford a very convenient basis for such investigations, and inasmuch as the present work is a contribution to such studies, its object will have been attained.

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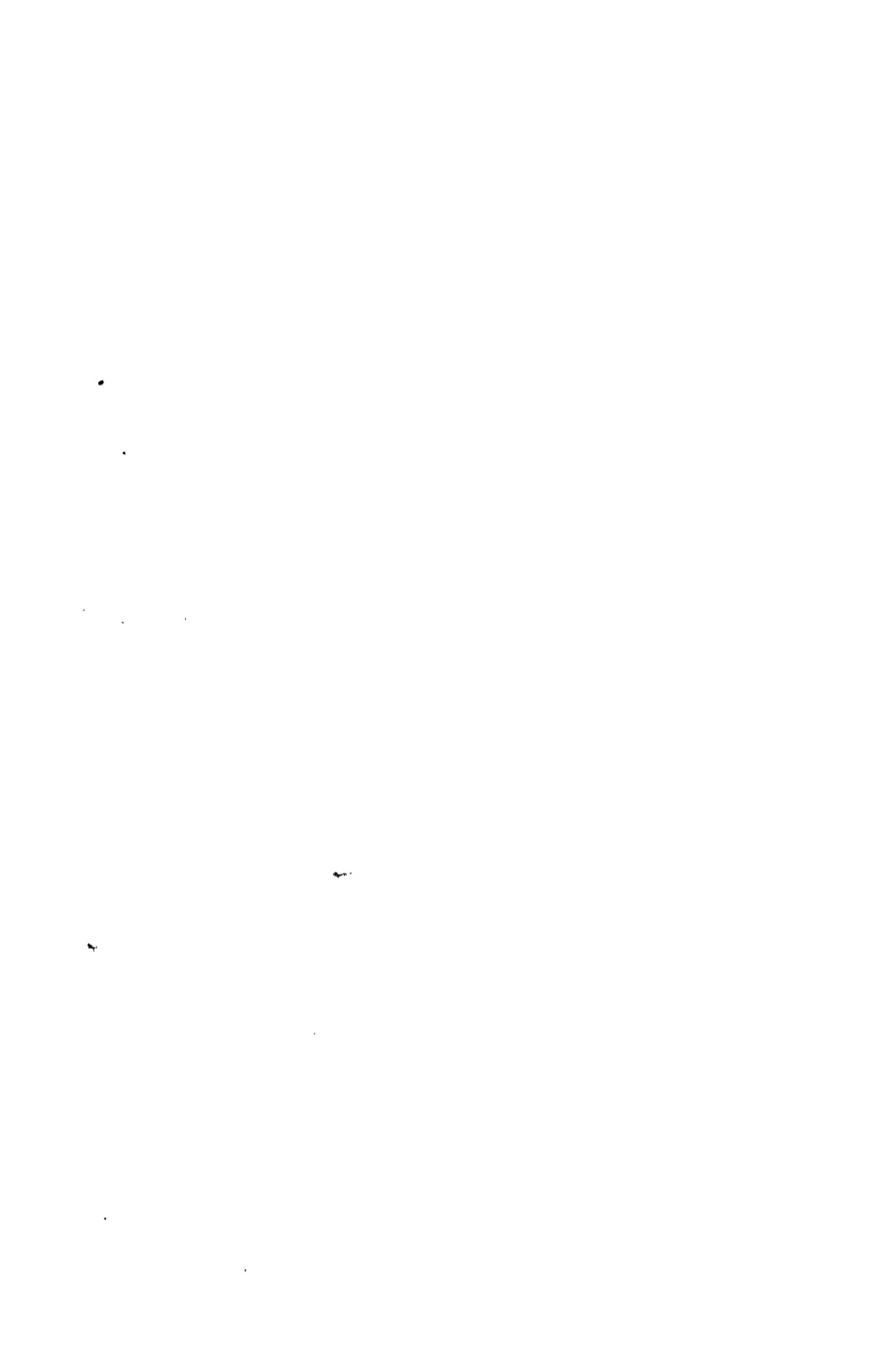
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